ABSTRACT BOOK

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

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

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Mr. Sylvain Gervason [1], Dr. Thomas Botzanowski [2], Dr. Christina Sophia Müller [3], Prof. Volker Schünemann [3], Dr. Sarah Cianferani [4], Dr. Michel Toledano [5], Dr. Benoit D'Autreaux [5]

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

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

In mitochondria, Fe-S clusters are assembled by the Iron-Sulfur Cluster assembly (ISC) machinery, which comprises the scaffold protein ISCU on which Fe-S clusters are assembled, the cysteine desulfurase complex NFS1-ISD11-ACP providing sulfur as a cysteine-bound persulfide (Cys-SSH) and the ferredoxin 2 – ferredoxin reductase system FDX2 – FDXR supplying electrons. Fe-S cluster assembly is supposed to rely on confined production of sulfide in the vicinity of a still ill-defined iron center in ISCU, but this has never been demonstrated. Moreover, previous *in vitro* reconstitutions of the ISC machinery were shown to produce free sulfide that can bind free iron and promotes Fe-S cluster reconstitution, but this process is not considered physiologically relevant since sulfide production is not confined to ISCU. Thereby, the mechanism of Fe-S clusters assembly is still unclear, which has precluded breakdown of the assembly sequence into elementary steps to determine at which step FXN operates.

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

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

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

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

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

Dr. Grazia Cotticelli [1], ***Dr. Roberto Forestieri*** [2], ***Dr. Shujuan Xia*** [1], ***Mr. Taehee Lee*** [1], ***Dr. Donna Huryn*** [2], ***Prof. Amos Smith III*** [2], ***Prof. Robert Wilson*** [1]

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Friedriech ataxia (FRDA) is characterized by a progressive neurodegeneration. Most patients develop a cardiomyopathy in which hypertrophy is accompanied by a replacement of cardiomyocytes with fibrotic tissue. The molecular mechanisms leading to cardiomyocyte cell death are incompletely understood.

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

We have previously shown that cell lines commonly used to study FRDA are sensitive to erastin and RSL-3, both known inducers of ferroptosis, and, conversely, ferroptosis inhibitors are efficacious in protecting human and mouse cellular models of FRDA treated with ferric ammonium citrate (FAC) and an inhibitor of glutathione synthesis (BSO). FRDA cells have long been known to be sensitive to treatment with iron, BSO, or a combination of both. Moreover, the ferroptosis inhibitor SRS11-92 decreased the cell death associated with frataxin knockdown in healthy human fibroblasts. Taken together, these data suggested activation of the ferroptosis pathway in FRDA cells and, consequently, the possibility that ferroptosis inhibitors could be used as therapeutics in Friedreich ataxia.

Oleic acid is an eighteen carbon monounsaturated omega-9 fatty acid, that has been reported to inhibit imidazole-ketone-erastin induced ferroptosis in G-401 cells through an uncharacterized mechanism. We tested oleic acid in primary, patient-derived fibroblasts, as well as mouse cells with FRDA-associated mutations treated with FAC and BSO and found that it was efficacious in rescuing cell viability.

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

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

Druggable genome screen identifies new regulators of the abundance and toxicity of ATXN3, the Machado-Joseph disease protein

Thursday, 14th November - 12:00: Mechanism of Disease - Oral Abstracts Part 1 - Abstract ID: 230

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 230

Ms. Naila S. Ashraf [1], Dr. Joanna R. Sutton [2], Dr. Yemen Yang [1], Mr. Bedri Ranxhi [2], Dr. Kozeta Libohova [2], Ms. Emily D. Shaw [1], Ms. Anna J. Barget [1], Dr. Sokol V. Todi [3], Dr. Henry L. Paulson [1], Dr. Maria do Carmo Costa [1]

1. *Department of Neurology, Michigan Medicine, University of Michigan,* ***2.*** *Department of Pharmacology, Wayne State University School of Medicine,* ***3.*** *Department of Pharmacology, Department of Neurology, Wayne State University School of Medicine*

Background: Machado-Joseph disease (MJD, also known as Spinocerebellar ataxia type 3) is a neurodegenerative disorder caused by a CAG repeat expansion encoding an abnormally long polyglutamine (polyQ) tract in the disease protein, ataxin-3 (ATXN3). No preventive treatment is yet available for MJD. Because MJD is likely caused by a toxic gain of ATXN3 function, a rational therapeutic strategy is to reduce mutant ATXN3 levels by targeting pathways that control its production or stability. Here, we sought to identify genes that modulate ATXN3 levels as potential therapeutic targets in this fatal disorder.

Methods: We screened a collection of siRNAs targeting 2742 druggable human genes using a cell-based assay based on luminescence readout of polyQ-expanded ATXN3. From 317 candidate genes identified in the primary screen, 100 genes were selected for validation. Among the 33 genes confirmed in secondary assays, 15 were validated in an independent cell model as modulators of pathogenic ATXN3 protein levels. Ten of these genes were then assessed in a *Drosophila* model of MJD, and one was confirmed as a key modulator of physiological ATXN3 abundance in MJD neuronal progenitor cells.

Results: Among the 15 genes shown to modulate ATXN3 in mammalian cells, orthologs of *CHD4*, *FBXL3*, *HR* and *MC3R* regulate mutant ATXN3-mediated toxicity in fly eyes. Further mechanistic studies of one of these genes, *FBXL3*, encoding a F-box protein that is a component of the SKP1-Cullin-F-box (SCF) ubiquitin ligase complex, showed that it reduces levels of normal and pathogenic ATXN3 in MJD neuronal progenitor cells, primarily via a SCF complex-dependent manner. Bioinformatic analysis of the 15 genes revealed a potential molecular network with connections to tumor necrosis factor-α/nuclear factor-kappa B (TNF/NF-kB) and extracellular signal-regulated kinases 1 and 2 (ERK1/2) pathways.

Conclusions: We identified 15 druggable genes with diverse functions to be suppressors or enhancers of pathogenic ATXN3 abundance. Among identified pathways highlighted by this screen, the FBXL3/SCF axis represents a novel molecular pathway that regulates physiological levels of ATXN3 protein.

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

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

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

Dr. Valentine Mosbach [1], ***Dr. Alain Martelli*** [2], ***Mrs. Nadège Diedhou*** [1], ***Mrs. Aurelie Eisenmann*** [1], ***Dr. Béatrice Py*** [3], ***Dr. Sandrine Ollagnier de Choudens*** [4], ***Prof. Helene Puccio*** [5]

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

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

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

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

Ms. Layne Rodden [1], ***Ms. Kaitlyn Gilliam*** [1], ***Ms. Christina Lam*** [1], ***Dr. Sahar Al-Madhawi*** [2], ***Dr. Mark Pook*** [3], ***Dr. Sanjay Bidichandani*** [1]

1. *University of Oklahoma HSC,* ***2.*** *Brunel University London,* ***3.*** *Brunel*

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

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

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

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

Ms. ELIZABETH MERCADO-AYON [1], ***Mr. Nathan Warren*** [2], ***Dr. David Lynch*** [3], ***Dr. Hong Lin*** [4]

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Introduction:

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

Methods:

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

Results:

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

Conclusion:

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

A novel AAV-based miQURE gene therapy for SCA3

Thursday, 14th November - 16:10: Therapeutic Approaches and Drug Discovery - Oral Abstracts Part 1 - Abstract ID: 95

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 95

Dr. Lodewijk Toonen [1], Dr. Rui Jorge Nobre [2], Ms. Eva Haas [3], Dr. Janice Stricker-Shaver [3], Dr. Jeannette Hübener-Schmid [3], Ms. Cynthia Brouwers [1], Mr. Raygene Martier [1], Dr. Astrid Vallès [1], Dr. Joseph Higgins [4], Prof. Sander van Deventer [1], Prof. Huu Nguyen [5], Prof. Luis Pereira de Almeida [2], Dr. Pavlina Konstantinova [1], Dr. Melvin Evers [1]

1. *uniQure B.V.,* ***2.*** *Center for Neuroscience and Cell Biology, University of Coimbra,* ***3.*** *Institute of Medical Genetics and Applied Genomics, University of Tübingen, Tübingen,* ***4.*** *uniQure inc.,* ***5.*** *Department of Human Genetics, Ruhr University Bochum*

Spinocerebellar ataxia type 3 (SCA3), or Machado-Joseph disease (MJD), is a fatal neurodegenerative disorder characterized by brainstem and cerebellar atrophy. Clinical manifestations predominantly include progressive gait ataxia with the involvement of cranial nerves. An expansion of a CAG trinucleotide repeat in the ataxin-3 gene (*ATXN3*) causes the accumulation of aberrant, toxic ataxin-3 protein in brain regions located in the posterior fossa. Lowering the expression of the responsible *ATXN3* gene should result in alleviation of mutant protein toxicity.

A non-allele-specific *ATXN3* silencing approach was investigated using the proprietary, next-generation miQURE™ technology. Artificial therapeutic microRNAs were engineered to target various regions of the *ATXN3* gene (mi*ATXN3*). The mi*ATXN3* candidates were screened *in vitro* for their silencing efficacies by using a luciferase reporter co-expressing *ATXN3*. Three mi*ATXN3* candidates were selected for further testing and packaged into AAV (AAV-mi*ATXN3*).

The AAV-mi*ATXN3* candidates were tested for target engagement and potential off-target activity in neurons differentiated from induced pluripotent stem cells (iPSCs). Small RNA sequencing showed efficient guide strand processing without any passenger strands. The AAV-mi*ATXN3* candidates strongly reduced *ATXN3*mRNA in the iPSCs-derived neurons.

In vivo reduction of mutant ataxin-3 was tested in a SCA3 knock-in mouse model by intraventricular, intracisternal and cerebellar intraparenchymal AAV5-mi*ATXN3* injections. Intracisternal AAV-mi*ATXN3*administration resulted in the most effective reduction (up to 65%) of mutant ataxin-3 protein in the cerebellum and brain stem of SCA3 mice. Next, proof-of-concept of pathology improvement upon ataxin-3 lowering was shown in a lentiviral SCA3 mouse model. Intra-striatal administration of AAV-mi*ATXN3*resulted in a strong reduction of mutant ataxin-3 production and prevention of toxic ataxin-3 aggregation

In conclusion, intracisternal administration of AAV-mi*ATXN3*significantly lowers mutant ATXN3 protein in the primary sites of SCA3 neuropathology. These results provide evidence to further investigate the distribution, efficacy, tolerability, and safety of AAV-mi*ATXN3* in larger animals.

Gluten ataxia: 24 years experience in the diagnosis and management of 600 patients

Thursday, 14th November - 16:25: Therapeutic Approaches and Drug Discovery - Oral Abstracts Part 1 - Abstract ID: 250

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 250

Prof. Marios Hadjivassiliou [1], ***Prof. Nigel Hoggard*** [2], ***Dr. Priya Shanmugarajah*** [1], ***Dr. Panagiotis Zis*** [1], ***Dr. Ptolemaios Sarrigiannis*** [1], ***Dr. Richard Grunewald*** [1], ***Prof. David Sanders*** [1]

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Introduction

The term gluten ataxia (GA), introduced in 1998 by our group, describes patients with an immune mediated ataxia characterized by positive antigliadin antibodies (AGA) and progressive cerebellar ataxia in the absence of alternative etiology. The diagnosis of GA remains problematic for a number of reasons: some neurologists still consider such a diagnosis controversial and thus patients are not being tested for it and are deprived of this diagnosis, there is limited availability of AGA testing, there is variability in the performance of commercial AGA assays and finally there is confusion regarding enteropathy (coeliac disease-CD) being a prerequisite for the diagnosis. We present our 24-year experience in diagnosing and managing 600 patients with GA.

Methods

The cohort consisted of patients from all over the UK. All patients were referred, seen, treated and followed up at regular intervals at the Sheffield Ataxia Centre (one of only 2 National Ataxia Centres in the UK). All patients were tested for AGA, transglutaminase 2 (TG2) and endomysium (EMA) antibodies. Testing for transglutaminase 6 antibodies was done on a research basis. All patients positive for AGA (with or without TG2 and/or EMA antibodies) were offered gastroscopy and duodenal biopsy. The presence of CD was defined by the triad of villous atrophy, crypt hyperplasia and increased intraepithelial lymphocytes on histological examination of the duodenal mucosa. All patients positive for AGA were referred to experienced dietitians for advice regarding strict gluten-free diet (GFD) irrespective of the presence of enteropathy. All patients underwent MRI including MR spectroscopy of the cerebellum (vermis and right hemisphere) at baseline and at 1 year after strict GFD. Serological testing for all the antibodies was repeated at 6 monthly intervals to establish adherence to GFD. Further dietetic review was offered for those patients where antibodies were still positive after a year on GFD.

Results

In 88% of all the patients diagnosed with GA the diagnosis was made after being referred and tested at the Sheffield Ataxia Centre. By definition all 600 patients with GA were positive for AGA. The prevalence of GA was 20% amongst all ataxias and 51% amongst idiopathic sporadic ataxias. Of 600 GA patients assessed, 55% were female. Mean age at presentation was 52 (range 16-95) and mean duration of ataxia was 13 years. Mild ataxia (walk unaided) affected 74%, moderate ataxia (walking aid) 18% and severe ataxia (wheelchair bound) 8%. Enteropathy was found in 47%. MR spectroscopy of the cerebellum showed vermian involvement in 100% with much less involvement of the cerebellar hemispheres in contrast to what is seen in genetic ataxias. EMA and TG2 antibodies were only positive in 47% of patients and were thus not sufficient to diagnose GA as 53% of patients without enteropathy were negative for these antibodies. 73% of patients with GA (with and without enteropathy) had antibodies against TG6, a new diagnostic marker for this disease. Patients with GA who strictly adhered to a GFD (with elimination of AGA) showed improvement of the ataxia on clinical and MR spectroscopy assessments. The commonest reason for lack of response was poor adherence to GFD. A limited number of patients who were very strict with their GFD

required immunosuppression with mycophenolate. Rarely patients were found to have refractory CD type 2. The neurological phenotype in refractory CD was that of ataxia associated with cortical myoclonus which tended to be progressive despite immunotherapy.

Conclusions

GA is the commonest cause of sporadic ataxia and one of the few treatable ataxias. The diagnosis relies on the use of native AGA testing. Despite this it remains largely undiagnosed resulting in progressive disability and irreversible cerebellar degeneration. A high index of suspicion, better recognition and access to appropriate diagnostic serological assays are imperative in order to make an early diagnosis and avoid permanent disability.

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

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

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

Dr. Michael Clark [1], ***Ms. Manogna Chintala*** [2], ***Mrs. Melanie Pride*** [2], ***Prof. Ronald Payne*** [2]

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

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

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

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

Allele-specific and Non-invasive AAV-based silencing of mutant ataxin-3 alleviates neuropathology and motor deficits in Spinocerebellar ataxia type 3

Thursday, 14th November - 16:55: Therapeutic Approaches and Drug Discovery - Oral Abstracts Part 1 - Abstract ID: 284

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 284

Dr. Rui Jorge Nobre [1], Ms. Joana Saraiva [2], Ms. Clelia Fusco [2], Ms. Susana Paixão [2], Dr. Catarina Miranda [2], Dr. Magda Santana [2], Prof. Miguel Sena-Esteves [3], Prof. Luis Pereira de Almeida [1]

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Machado-Joseph disease (MJD) or Spinocerebellar ataxia type 3 is a dominantly-inherited neurodegenerative disease. It affects mostly the cerebellum and is the most common dominantly inherited ataxia worldwide. It is associated with the expansion of a (CAG)n tract in the coding region of the causative gene MJD1/ATXN3, which encodes ataxin-3, a polyubiquitin-binding protein involved in ubiquitin-mediated proteolysis. The abnormal over-repetition of the CAG trinucleotide is translated into an expanded polyglutamine tract within ataxin-3, conferring toxic properties to this protein, resulting in severe clinical features and leading to death.

Although there is no cure, it has been shown that RNA interference (RNAi) holds great promise for its treatment. RNAi has been employed to target both mutant and non-mutant ataxin-3 alleles. Nevertheless, it is unknown whether neuronal cells in the human brain will tolerate long-term silencing of wild-type ataxin-3. With the aim of translation to clinics, we aimed at developing an AAV-based microRNA gene therapy that enables an allele-specific silencing of mutant ataxin-3 and alleviation of MJD upon intracranial or intravenous injection.

Specific gene silencing RNAs, whose anti-sense sequences are complementary to SNPs that are in linkage disequilibrium with the disease-causing expansion, were firstly designed and tested in modified neuronal cell lines. An AAV9 vector encoding the most effective artificial microRNA (AAV9-mirATAX3) was then generated and validated in a lentiviral-based model of MJD upon intracranial injection. Finally, severely-impaired transgenic mice were intravenously-injected at postnatal day one (PN1), submitted to behavioral tests at three different time points, underwent Magnetic resonance imaging/spectroscopy (MRI/MRS) at PN75 and were sacrificed at PN95.

The silencing potential of the mirATX3 sequence demonstrated superior specificity *in vitro* compared to the silencing sequence previously reported. AAV9-mirATAX3's treatment reduced the number of protein aggregates and cerebellar neuropathology in both animal models and led to significant improvements in behavioral tests. Moreover, MRI/MRS data indicated that mirATXN3 treatment ameliorates the levels of a specific set of neurometabolites, which can be used as therapeutic biomarkers.

This study provides compelling evidence that AAV9-mirATAX3 is able to silence mutant ataxin-3 in different disease models, through different routes of administration. This may have a significant impact on the treatment of MJD, as well as other Polyglutamine diseases.

NPTX1 mutations cause reticulum endoplasmic stress and cerebellar ataxia

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 188

Friday, 15th November - 09:10: Genetics of Disease - Oral Abstracts - Abstract ID: 188

Dr. Marie Coutelier [1], Dr. Maxime Jacoupy [1], Dr. Alexandre Janer [2], Dr. Flore Renaud [3], Mr. François Ancien [4], Dr. Roxanne Larivière [2], Mr. Rémi Valter [1], Mr. Nicolas Sgarioto [2], Dr. Fabrizio Pucci [4], Dr. Marianne Rooman [4], Dr. Isabelle Le Ber [5], Dr. Perrine Charles [5], Dr. Alexis Brice [5], Dr. Eric Shoubridge [2], Dr. Dimitri Gilis [4], Prof. Alexandra Durr [5], Prof. giovanni Stevanin [1]

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Autosomal dominant spinocerebellar ataxias (SCA) are characterized by a marked genetic heterogeneity, with more than forty causative genes identified so far, and half the patients lacking a molecular diagnosis. In a large family with 9 symptomatic patients, we aimed at identifying the genetic cause of SCA by performing exome sequencing. We identified a G389R missense mutation in *NPTX1*, segregating in all affected. This mutation was recurrent in two independent pedigrees without a founder effect while another missense mutation, E327G, was identified in a smaller pedigree with two affected patients. The phenotype in these 4 families is a late onset (range 34-71 years) slowly progressive cerebellar ataxia, with myoclonic tremor, mild cerebellar atrophy on MRI, down beat nystagmus and cognitive cerebellar syndrome. Both mutations affect conserved amino-acid residues and are extremely rare or missing from public databases. NP1, the protein encoded by *NPTX1* localizes in the endoplasmic reticulum. We show that both mutations alter the organelle morphology and induce ATF6-mediated stress, ultimately leading to increased cell cytotoxicity in COS cells. The E327G change also abolishes the protein secretion, as well as the formation of NP1 complexes. Mass spectrometry demonstrated loss of interaction with endoplasmic reticulum proteins for this variant. In silico modeling of the complex evidenced a destabilizing effect for the E327G change, located at the interface between monomers. On the contrary, the G389 residue is located at the protein surface and has no effect on the complex stability. No phenotype-genotype correlation could be established. We hence suggest that mutations in *NPTX1* can lead to spinocerebellar ataxia via endoplasmic stress, via a dominant negative complex-destabilizing process, or via a direct alteration of the protein function.

High throughput sequencing-based genotyping of the CAG repeat structure at the spinocerebellar ataxia type 3 and 7 loci

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 18

Friday, 15th November - 09:25: Genetics of Disease - Oral Abstracts - Abstract ID: 18

Mr. Ahmed Sidky [1], ***Dr. Sarah Cumming*** [1], ***Dr. Marc Ciosi*** [1], ***Mr. Alastair Maxwell*** [1], ***Ms. Shaymaa Shurrab*** [1], ***Dr. Graham Hamilton*** [1], ***Prof. Darren Monckton*** [1]

1. *The university of Glasgow*

Spinocerebellar ataxia subtype 3 (SCA3), the most common subtype of the spinocerebellar ataxia, is caused by the expansion of a complex polyglutamine encoding repeat (CAG_2-CAAAAG-CAG$(CAA)_{0\text{-}1}$-CAG_n(G/C)GG) in the exon 10 of the *ATXN3*gene, where n varies from ~ 6 to 35 in the general population. The G>C SNP (*rs12895357*) is located directly downstream of the SCA3 (CAA/AAG/CAG) repeat. Although the C-SNP haplotype is relatively common in the general population, but it was observed to be mostly associated with the expanded disease-associated SCA3 repeats. This SNP was found to be helpful in specifically detecting and silencing the expression of the expanded SCA3 repeat alleles. SCA7 is caused by the expansion of another polyglutamine encoding repeat in exon 3 of the *ATXN7*gene. The typical SCA7 repeat structure is ($(CAG)_n$), where n varies from ~ 4 to 19 in the general population. Moreover, it is unlikely to observe alleles of repeat lengths between 20 and 26 CAG repeats in the general population. The CAG intermediate repeat length varies from ~ 28 to 35, and the disease associated repeat length varies from ~ 36 to 80. The genetic diagnosis of these disorders is achieved through capillary gel electrophoresis of PCR products. Although this method can provide accurate estimates of total repeat length, it doesn't reveal the precise repeat structure and rare variants. Furthermore, although the genetic test is very accurate, most patients take many years to achieve a clinical diagnosis prior to genetic testing. Thus, population screening of these loci could provide an early disease diagnosis for mildly affected and asymptomatic individuals. Early diagnosis could provide improved opportunities for making informed reproductive choices and enable new treatments to be used in a preventative manner. Thus, we developed a new high throughput next generation sequencing approach for the diagnosis and population screening of these loci. We screened DNA samples for individuals from the general Scottish population. A group of 364 individuals were genotyped for the SCA3 locus and 361 individuals for the SCA7 locus. We managed to accurately measure the repeat lengths of the repeats, in addition to provide an exact description of the (CAA/AAG/CAG) repeat of SCA3 and the (CAG/CCG) repeat of SCA7. In the same assay, we were also able to genotype the flanking G>C SNP (rs12895357), that further improved the accuracy of genotyping and enhanced the variation between haplotypes at the level of the general population. In addition, we are the first who observed the variation of the CCG repeat, that is located directly downstream the SCA7 CAG repeat so the repeat structure should be ($(CAG)_n(CCG)_{2\text{-}3}$). Our sequencing-based results are much more informative for the repeat structure variation, revealing a higher frequency of heterozygosity. These data support a move toward sequence based genetic diagnosis and population screening for the SCAs.

NOVEL GENETIC MODIFIERS OF MACHADO-JOSEPH DISEASE (MJD/SCA3) IDENTIFIED BY WHOLE-EXOME SEQUENCING

Friday, 15th November - 09:40: Genetics of Disease - Oral Abstracts - Abstract ID: 32

Ms. Mafalda Raposo [1], ***Ms. Ana Rosa Vieira Melo*** [1], ***Ms. Daniela Vilasboas-Campos*** [2], ***Ms. Ana Filipa Ferreira*** [1], ***Dr. João Vasconcelos*** [3], ***Dr. Isabel Alonso*** [4], ***Dr. Paulo Silva*** [4], ***Prof. Laura Jardim*** [5], ***Prof. Maria Luiza Saraiva-Pereira*** [6], ***Dr. Conceição Bettencourt*** [7], ***Prof. Henry Houlden*** [8], ***Prof. Jorge Sequeiros*** [9], ***Prof. Peter Heutink*** [10], ***Prof. Patricia Maciel*** [2], ***Prof. Manuela Lima*** [1]

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Introduction: Machado-Joseph disease (MJD/SCA3) is a neurodegenerative polyglutamine disorder exhibiting marked clinical heterogeneity. The size of the (CAG)n at *ATXN3*, the causative locus, explains only ~50% of the variation in age at onset (AO), suggesting that other factors, namely genetic, contribute to it. Identification of genetic modifiers will allow a better prediction of AO, with translational value to genetic counselling and trials stratification, and may lead to the proposal of new targets for therapeutic interventions. To identify novel genetic modifiers, we performed whole-exome sequencing (WES) in a discovery cohort of AO-concordant and AO-discordant pairs of first-degree relative patients from the homogeneous Azorean MJD cohort.

Materials and Methods: WES was performed using DNA samples from 4 concordant and 4 discordant pairs. Patients of the AO-discordant pairs showed an average AO difference of 9 years; patients of AO-concordant pairs presented an average AO difference of 2 years. Both members of each pair of patients presented with the same (CAG)n size. Variants for which the genotype differed in discordant pairs and was the same in concordant pairs were selected for genotype-phenotype correlations and tested in a sample of 253 MJD patients. In parallel, we determine the functional role of the genes harbouring frameshift and missense variants and e modulated the expression of those genes in a MJD *C. elegans* transgenic model by RNAi.

Results: Ninety-two variants in 52 genes were analysed. Gene enrichment analyses resulted in 18 over-represented pathways, including the nervous system-related neuroregulin signalling and agrin interactions at neuromuscular junctions. In the Portuguese-Brazilian cohort (N=253), four variants in three genes were significantly associated with a later AO: *DIDO1, ACTG1*and *CFAP57*. Silencing of *SIM1, ASPSCR1, EP300, GLI4, PYROXD and DCLRE1C C. elegans* orthologues significantly restored the locomotion of this transgenic model alongside with a reduction in the number of ataxin-3 aggregates.

Conclusions: WES allowed the identification of novel modifier genes by delaying AO of MJD patients and by amelio-

rated the phenotype of a *C. elegans* model of MJD. Additional studies targeting these modifier genes and pathways will reveal their potential in disease-modifying therapies.

The Structure of the Human ACP-ISD11 Heterodimer

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 140

Friday, 15th November - 10:30: Therapeutic Approaches and Drug Discovery - Oral Abstracts Part 2 - Abstract ID: 140

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In recent years the mammalian mitochondrial protein complex for iron-sulfur cluster assembly has been the focus of major studies. This is partly because of its high relevance in cell metabolism, but also because mutations of the involved proteins are the cause of several human diseases. Cysteine desulfurase NFS1 is the key enzyme of the complex and the active form of NFS1 is stabilized by the small protein ISD11. In this work, the structure of the human mitochondrial ACP-ISD11 heterodimer was solved at 2.0 Å resolution. ACP-ISD11 forms a cooperative unit. The 4'-phosphopantetheine-acyl chain, which is covalently bound to ACP, interacts with several residues of ISD11, modulating together with ACP the foldability of ISD11. Recombinant human ACP-ISD11 interacted with the NFS1 desulfurase, thus yielding an active enzyme, and the core complex NFS1/ACP-ISD11 was activated by frataxin and ISCU proteins. Motions of ACP-ISD11 dimer were investigated by molecular dynamics simulations, showing the persistence of the interactions between both protein chains. The conformation of the dimer is similar to the one found in the context of the supercomplex core $(NFS1/ACP\text{-}ISD11)_2$, which contains the *E. coli* ACP instead of the human variant. This fact suggests a sequential mechanism for supercomplex consolidation, in which the ACP-ISD11 complex may fold independently and after that, the NFS1 dimer is stabilized.

End targeting oligonucleotides increase stability of FXN mRNA

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

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

Dr. Marek Napierala [1], ***Dr. Yanjie Li*** [1], ***Dr. Jun Wang*** [1], ***Dr. David lynch*** [2], ***Dr. Xiulong Shen*** [3], ***Dr. David Corey*** [3], ***Dr. Caroline Woo*** [4], ***Dr. Jonathan Cherry*** [4], ***Dr. Jill Napierala*** [1]

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

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

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

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

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

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

Understanding and therapeutically targeting mGluR1-TRPC3-mediated spinocerebellar ataxias

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 104

Friday, 15th November - 10:30: Mechanism of Disease - Oral Abstracts Part 2 - Abstract ID: 104

Ms. Jodie Collingridge [1], Ms. Alaa Baazaoui [1], Dr. Kelly M. Gatfield [2], Dr. Andrew C. Pearce [2], Dr. John Liddle [2], Dr. Peter L. Oliver [3], Dr. Esther B. E. Becker [1]

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The spinocerebellar ataxias (SCAs) are a clinically and genetically heterogeneous group of neurodegenerative disorders characterized by the progressive dysfunction of the cerebellum. No effective treatments exist for these devastating diseases. A major challenge is to better understand the specific disease-causing mechanisms underlying this complex group of diseases and to identify common pathological pathways that could be targeted therapeutically.
Our work has uncovered a key role for the mGluR1-TRPC3 pathway in spinocerebellar ataxia. We identified the first patients and families harboring dominant mutations in the *TRPC3*and *GRM1*genes, causing SCA41 and SCA44, respectively. Using genome editing, we have recently created the first mouse model of SCA44 that harbors a gain-of-function patient mutation in the *Grm1* gene encoding the metabotropic glutamate receptor mGluR1. Here, we will present on the cellular and behavioral phenotypes of this novel disease model.
Importantly, disturbed mGluR1-TRPC3 signaling is also linked to several other genetically distinct forms of SCAs, thus making it a strong candidate for a commonly affected disease pathway in cerebellar ataxia. We will present our work identifying compounds that could be used as potential therapeutics targeting this central disease pathway.

REINSTATING LEVELS OF DYSREGULATED AUTOPHAGY-ASSOCIATED TRANSCRIPTS ALLEVIATES NEUROPATHOLOGY AND MOTOR IMPAIRMENTS OF MOUSE MODELS OF MACHADO-JOSEPH DISEASE

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 273

Friday, 15th November - 10:45: Mechanism of Disease - Oral Abstracts Part 2 - Abstract ID: 273

Ms. Ana Ferreira [1]***, Ms. Inês Martins*** [2]***, Ms. Diana Lobo*** [3]***, Ms. Dina Pereira*** [4]***, Mr. Miguel Lopes*** [3]***, Ms. Sara Lopes*** [5]***, Ms. Rosário Faro*** [5]***, Dr. Dineke Verbeek*** [6]***, Dr. Thorsten Schmidt*** [7]***, Prof. Clévio Nóbrega*** [8]***, Dr. Luís Pereira de Almeida*** [1]

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Machado-Joseph disease (MJD) or spinocerebellar ataxia type 3 (SCA3), is a late onset neurodegenerative disorder that, although rare, is the most common autosomal dominantly inherited ataxia worldwide. Causative of MJD is an expansion of a CAG trinucleotide repeat in the *ATXN3/MJD1* gene that is translated into an expanded polyglutamine in the corresponding ataxin-3 (ATXN3) protein, which becomes prone to aggregation. The presence of intranuclear inclusions correlates with disease progression and dysfunction of cell quality control systems, as is the case of the autophagy pathway. In fact, previous observations have implicated autophagy defects in the accumulation of mutATXN3 aggregates and neurodegeneration in different models of MJD (Nascimento-Ferreira et al., 2011; Onofre et al., 2016; Sitller et al., 2018). Nevertheless, how dysregulation of the autophagy pathway occurs in MJD, particularly at the transcriptional level was not yet investigated. Therefore, the main goal of this project was to investigate transcriptional modifications of the autophagy pathway in MJD models and to interfere with the levels of affected transcripts aiming at reestablishment of efficient autophagic clearance of misfolded and aggregated ATXN3.

First, we thoroughly characterized the autophagy-related transcriptional dysregulation in several models of the disease, providing evidence of different pathways wherein autophagy is impaired in MJD. Interestingly, we detected a robust decrease of ULK1 and ULK2 expression, and ULK1 seems to be intimately associated with the disease progression in a transgenic mouse model of MJD. We next evaluated ULK1 and ULK2 as a potential therapeutic targets. *In vitro*, upon ULK1 and both transcripts over-expression, we observed a decrease in mutATXN3 protein levels. The same beneficial effect on the clearance of ATXN3 toxic species was observed *in vivo* after transduction of mice striata with vectors encoding ULK1/2. Finally, in a transgenic mouse model, we found that the reestablishment of ULK1/2 levels led to the amelioration of behaviour motor performance and disease-associated neuropathology, suggesting that ULK1/2 plays an important role in reestablishment of the correct function of the autophagy pathway.

In conclusion, in this work, we elucidated impairments within the mechanics of autophagy in MJD and identified new promising targets for the treatment of this incapacitating disease.

The *in vivo* experiments were carried out in accordance with the European Community directive (2010/63/EU) for the care and use of laboratory animals. Supported by the ERDF through the Regional Operational Program Center

2020, Competitiveness Factors Operational Program (COMPETE 2020) and National Funds through FCT (Foundation for Science and Technology) - BrainHealth2020 projects (CENTRO-01-0145-FEDER-000008), ViraVector (CENTRO-01-0145-FEDER-022095), CortaCAGs (POCI-01-0145-FEDER-016719), SpreadSilencing POCI-01-0145-FEDER-029716, as well as SynSpread, ESMI and ModelPolyQ under the EU Joint Program - Neurodegenerative Disease Research (JPND), the last two co-funded by the European Union H2020 program, GA No.643417; by National Ataxia Foundation (USA), the American Portuguese Biomedical Research Fund (APBRF) and the Richard Chin and Lily Lock Machado-Joseph Disease Research Fund. A.C.V.F. is supported by the FCT fellowship SFRH/BD/87804/2012.

Targeting altered proteostasis in cellular and mouse models of AFG3L2-related cerebellar ataxias (SCA28 and SPAX5)

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 177

Friday, 15th November - 11:00: Mechanism of Disease - Oral Abstracts Part 2 - Abstract ID: 177

Dr. Andrea Del Bondio [1], ***Dr. Valentina Baderna*** [1], ***Dr. Susanna Tulli*** [1], ***Prof. Giorgio Casari*** [2], ***Dr. Francesca Maltecca*** [1]

1. *Ospedale San Raffaele, Milan,* ***2.*** *Telethon Institute of Genetics and Medicine*

Heterozygous mutations in AFG3L2 cause Spinocerebellar Ataxia type 28 (SCA28), while homozygous mutations in the same gene result in severe childhood spastic-ataxia (SPAX5).

AFG3L2 is a mitochondrial protease which assembles in homo- and hetero-oligomers with paraplegin (the *m*-AAA complexes), exerting crucial protein quality control in the inner mitochondrial membrane.

Altered mitochondrial dynamics is central to the pathogenesis of these diseases. We indeed observed mitochondrial fragmentation in *Afg3l2*$^{-/-}$ MEFs, SCA28 patient fibroblasts and *Afg3l2*$^{-/-}$ and *Afg3l2*$^{+/-}$ Purkinje cells,*in vivo* and *in vitro*. This phenomenon is caused by enhanced processing and turnover of the long isoforms of OPA1 (L-OPA1), the active mediators of inner mitochondrial membrane fusion. We found that over-activation of the inner membrane stress-sensitive metalloprotease OMA1 is responsible for the increased cleavage of L-OPA1 when AFG3L2 is mutated or absent. Subsequently, OMA1 undergoes autocatalysis as a self-limiting mechanism to allow restoration of L-OPA1.

We disclosed that OMA1 activation in our models is caused by proteotoxic stress due to the accumulation of mitochondria-encoded proteins, secondary to impaired AFG3L2-mediated quality control. Indeed, we demonstrated that accumulation of specific mitochondria-encoded proteins (i.e. ND1 and ATP6) elicits a mitochondrial proteotoxic stress response in the cerebellum of *Afg3l2*$^{-/-}$ mice, with upregulation of mitochondrial matrix chaperones. Notably, ND1 accumulation directly correlates with OMA1 over-activation and hampers the assembly of respiratory chain complex I in Afg3l2-depleted models. Mitochondrial proteotoxicity also triggers an integrated stress response, as demonstrated by upregulation of key mediators of this pathway. Consistent with this mechanism, the attenuation of mitochondrial protein synthesis results in stabilized OMA1 and OPA1 levels and restores mitochondrial tubulation in *Afg3l2*$^{-/-}$ cells and SCA28 patient fibroblasts. Moreover, preliminary results suggest that this approach improves mitochondrial transport in dendrites of primary *Afg3l2-/-* PCs.

Overall, our data improve the current knowledge of AFG3L2-related neurodegeneration, disclosing mitochondrial and cellular proteotoxicity as new potential therapeutic targets.

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

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

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

Mr. Giussepe Yañez [1], Dr. Anna Stepanova [2], Dr. Joriene de Nooij [3], Dr. Jordi Magrane [1]

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

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

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

Regulation of neuronal mRNA splicing and Tau isoform ratio by ATXN3 through deubiquitylation of splicing factors

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 211

Friday, 15th November - 12:05: Cellular and Animal Models of Disease - Oral Abstracts - Abstract ID: 211

Dr. Andreia Neves-Carvalho [1], Dr. Sara Duarte-Silva [2], Dr. Joana Silva [1], Dr. Bruno Almeida [1], Dr. Sasja Heetveld [3], Dr. Ioannis Sotiropoulos [2], Prof. Peter Heutink [4], Prof. Ka Wan Li [5], Prof. Patricia Maciel [1]

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The ubiquitylation/deubiquitylation balance in cells is maintained by Deubiquitylating enzymes, including ATXN3. The precise role of this protein, mutated in SCA3, remains elusive, as few substrates for its deubiquitylating activity were identified. Therefore, we characterized the ubiquitome of neuronal cells lacking ATXN3, and found altered polyubiquitylation in a large proportion of proteins involved in RNA metabolism, including splicing factors. Using transcriptomic analysis and reporter minigenes we confirmed that splicing was globally altered in these cells. Among the targets with altered splicing was SRSF7 (9G8), a key regulator of MAPT (Tau) exon 10 splicing. Loss-of-function of ATXN3 led to a deregulation of MAPT exon 10 splicing resulting in a decreased 4R/3R-Tau ratio. Similar alterations were found in the brain of a SCA3 mouse and humans, pointing to a relevant role of this mechanism in SCA3, and establishing a previously unsuspected link between two key proteins involved in different neurodegenerative disorders.

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

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

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

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

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

Introduction

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

This prospective longitudinal study aims to validate and evaluate the capacity of a new upper limb measure to both quantify upper limb impairment and capture change in function over a 20 and 40 week period in 40 individuals with FRDA.

Methods

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

Results

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

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

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

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

Conclusion

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

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

[1]Grice KO, et al., *Am J Occup Ther*. 2003; 57:570-3. [2]Tezenas du Montcel S. et al., *Brain*, 2008; 131:1352-1361. [3]Subramony SH, et al., *Neurology*2005;64: 1261–1262. [4]Trouillas P, et al., *J Neurol Sci* 1997, 145:205-211. [5]Schmitz-Hubsch T, et al., *Neurology*, 2006, 66:1717-1720.

Clinical scales and vestibulo-ocular reflex show changes in time since the pre-clinical phases in Machado-Joseph disease/spinocerebellar ataxia type 3 (BIGPRO Study)

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 260

Friday, 15th November - 15:25: Natural History, Biomarkers and Endpoints - Oral Abstracts Part 1 - Abstract ID: 260

Ms. Camila Oliveira [1], ***Ms. Gabriela Bolzan*** [1], ***Ms. Gabriela Ecco*** [1], ***Ms. Amanda Henz*** [1], ***Ms. Anastacia Rocha*** [1], ***Ms. Nathalia Kersting*** [1], ***Mrs. Mariana Rieck*** [2], ***Ms. Ana Carolina Martins*** [1], ***Prof. Vanessa Bielefeldt Leotti*** [2], ***Prof. Maria Luiza Saraiva-Pereira*** [2], ***Prof. Laura Jardim*** [2]

1. *UFRGS,* ***2.*** *HCPA & UFRGS*

Background and Objective

Spinocerebellar Ataxia Type 3/Machado-Joseph Disease (SCA3/MJD) is an autosomal dominant disorder caused by a CAG repeat expansion (CAGexp) at the ATXN3. Causal treatment is not available yet. During recent years, promising progress has been made in the understanding of the pathogenesis. Considering that a causal therapy will probably be more efficient if started early in life, reliable biomarkers for pre-clinical stages are needed. BIGPRO (Biomarkers and genetic modifiers in a study of presymptomatic and symptomatic SCA3/MJD carriers) is a longitudinal study aiming to validate biomarkers for disease progression in SCA3/MJD since pre-clinical periods (bigpro.webnode.com). We report baseline findings obtained from clinical scales and vestibulo-ocular reflex (VOR) measured by video-oculography from the 95 first individual participants.

Methods

Recruitment occurred between August 2017 and November 2018. Baseline data on clinical scales and oculomotor neurophysiology were collected from 95 subjects – 36 symptomatic and 59 at 50% risk for SCA3/MJD. Age at onset (AO) was considered the age at which the subject and her/his relatives first noticed gait ataxia. Time after onset was considered the time elapsed since the AO for each symptomatic subject. Genetic tests performed in at risk subjects were double-blind. For presymptomatic carriers (SARA < 3), the average time left until the onset of gait ataxia was called “time to onset”. The CAGexp was used to estimate time to onset both at birth and corrected by age, as described elsewhere (doi: 10.1111/ene.13779). Presymptomatic subjects were classified according to the predicted age at onset at birth and divided into two groups: asymptomatics far from age of onset (AFF) – when predicted to start symptoms in more than 4 years – or near (AN) age of onset – when disease start predicted to happen in 4 or less years. Time to/time after onset (TtoAfterOnset) was a unique dimension of time versus start of gait ataxia, estimated to all SCA3/MJD carriers.

Clinical outcome assessments (COAs) of interest for this report were the clinical scales (NESSCA, SARA, ICARS, INAScount, CCFS and SCAFI) and the vestibulo-ocular reflex (VOR), measured by video-oculography (EyeSeeCam - doi: 10.3233/VES-160579). The parameter of VOR chosen for comparisons was the regression of gain, a regression analysis of eye and head velocities between a time interval of 10ms before to 100ms after the onset of the impulse – the average of both sides (VORr). Parametric tests were performed when quantitative variables showed normal distributions. The statistical analysis program SPSS v.19.0 was used and results were considered statistically significant when $p<0.05$.

Results:

Thirty-seven of the 59 at 50% risk subjects were in fact SCA3/MJD carriers: 13 of them were at four or less years from their predicted AO. Overall characteristics of the 95 subjects, classified in four groups (symptomatic carriers,

AN, AFF and related controls) were shown in Table 1.

All parameters under study – NESSCA, SARA, ICARS, INAScount, SCAFI, CCFS and VORr – showed statistically significant differences when the four groups were studied. However, only NESSCA, INAScount, SCAFI and VORr results showed significant differences between controls and AN (**Table 2**).

TtoAfterOnset obtained from all 73 carriers of CAGexp correlated well ($p<0.0001$) with all clinical scales and VORr. When considering only asymptomatic carriers, time to onset correlated better with NESSCA, ICARS and VORr (rho=0.611, 0.635 and -0.537, $p<0.05$), but also with SARA and INAScount (rho= 0.355 and 0.445, $p<0.05$). VORr correlated significantly with all clinical scales, most strongly with ICARS ($r=0.747$, $p<0.001$) and NESSCA ($r=0.754$, $p<0.001$).

Discussion

VORr, INAScount, SCAFI and NESSCA distinguished pre-clinical carriers near the predicted age of onset. However, only VORr and NESSCA correlated well with time to onset when considering only pre-symptomatic carriers. In addition, VORr correlated well with validated clinical scales. These results suggest that VORr and NESSCA could be good candidates biomarkers for the pre-symptomatic period in SCA3/MJD. Longitudinal observations will deepen these observations and perhaps confirm these findings.

Acknowledgements: CAPES, CNPq, FAPERGS, FIPE-HCPA.

References: 10.1111/ene.13779: de Mattos et al. Age at onset prediction in spinocerebellar ataxia type 3 changes according to population of origin. *Eur J Neurol.* 2019 Jan;26(1):113-120. 10.3233/VES-160579: Luis et al. Vestibulo-ocular reflex dynamics with head-impulses discriminates spinocerebellar ataxias types 1, 2 and 3 and Friedreich ataxia. *J Vestib Res.* 2016 Jul 2;26(3):327-34.

Neurofilaments as blood biomarkers at the preataxic and ataxic stage of spinocerebellar ataxia type 3: a cross-species analysis in humans and mice

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 54

Friday, 15th November - 17:00: Natural History, Biomarkers and Endpoints - Oral Abstracts Part 2 - Abstract ID: 54

Dr. Carlo Wilke [1], Prof. Kathrin Reetz [2], Prof. Ludger Schöls [1], Prof. Thomas Klockgether [3], Dr. Christian Barrro [4], Dr. Jeannette Hübener-Schmid [5], Prof. Matthis Synofzik [1]

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also on behalf of the European Integrated Project on Spinocerebellar Ataxias (EUROSCA/RISCA) consortium, and the European Spinocerebellar Ataxia Type 3/Machado-Joseph Disease Initiative (ESMI)

Background. Spinocerebellar ataxia type 3 (SCA3) is a devastating multisystemic neurodegenerative disease for which targeted molecular therapies are coming into reach (e.g. antisense oligonucleotides). To pave the way for upcoming translational trials, easily accessible biomarkers in SCA3 are needed, particularly for subjects at the preataxic stage and validated also in animal models. We hypothesised that serum neurofilaments might serve as blood biomarkers of disease progression in both human SCA3 and mouse models, expecting increased concentrations already at the preataxic stage.

Methods. Serum neurofilament light (NfL) and phosphorylated neurofilament heavy (pNfH) levels were determined by ultra-sensitive single molecule array (Simoa) in cross-sectional samples of ataxic and preataxic SCA3 subjects and controls in two independent cohorts (ESMI cohort= cohort#1: n=160, EuroSCA/RISCA cohort = cohort#2: n=89). Serum NfL and pNfH were also assessed in a 304Q SCA3 knock-in mouse model across presymptomatic and symptomatic disease stages (n=147).

Findings. Ataxic SCA3 subjects showed increased serum NfL ($p<0.001$) and pNfH ($p<0.001$) levels in cohort #1, with NfL levels already increased in preataxic subjects ($p<0.001$). All of these results were replicated in cohort #2 (all $p<0.001$). Cross-sectional NfL levels correlated with clinical disease severity (Scale for the Assessment and Rating of Ataxia [SARA]; $r=0.46$, $p<0.001$; cohort #1) and with longitudinal disease progression (annual SARA score change, $\varrho=0.42$, $p=0.012$; cohort #2). CAG count and age were significant predictors of individual NfL concentrations (each $p<0.001$). NfL levels in preataxic subjects increased with proximity to individual expected onset of ataxia ($p<0.001$), with significant elevations already 7.5 years before onset. Serum NfL and pNfH increases in SCA3 humans were paralleled by similar changes in SCA3 knock-in mice, here also already starting at the presymptomatic stage (including pNfH increase) and close to the onset of ataxin-3 increase.

Interpretation. Serum concentrations of neurofilaments, particularly NfL, might provide easily accessible biomarkers of disease severity in ataxic and preataxic SCA3 subjects and mice prior to conversion. Neurofilaments thus entail potential applications as progression, onset/proximity and treatment-response markers in both human and murine SCA3 trials.

Funding: EU Joint Programme Neurodegenerative Disease Research, National Ataxia Foundation.

Extra-Mitochondrial Frataxin and Friedreich's Ataxia

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

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

Dr. Laurent Laboureur [1], Dr. Liwei Weng [1], Dr. Qingqing Wang [1], Ms. Lauren Hauser [2], Dr. David Lynch [2], Dr. Clementina Mesaros [1], Prof. Ian Blair [3]

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

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

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

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

Dr. Adam Vogel [1]

1. *The University of Melbourne / Redenlab*

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

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

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

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

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

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

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

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

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Background and Objectives

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

Methods

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

Results

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

- 50% of FA subjects had intramyocardial late gadolinium enhancement (LGE) on baseline CMR, consistent with local LV fibrosis. LGE typically occurred in the LV lateral wall and involved a small amount of LV myocardium (1.7±2.4 %). FA subjects with LGE showed greater LV concentric remodeling (mass/volume) and increased wall thickness than FA subjects without LGE. In areas without LGE, T1 mapping-derived parameters (markers of diffuse fibrosis) such as native T1 and extracellular volume (ECV) did not differ at baseline between groups. LGE, ECV, and native T1 were all unchanged at one-year follow-up.
- FA subjects performed maximal CPET based on HR increase, not significantly different from controls, meaning CPET values are interpretable and lower values in FA are explained by cardiovascular and/or muscular limitation (baseline visit HR at VO2 max 146±14 and 156±18 bpm in FA vs controls). Change over one year in FA was driven by decreased workload (work VO2 max 58.1±21.5 at baseline and 44.3±19.1 watts at one-year, whereas peak VO2 was maintained (911.6±338.4 at baseline and 894.4±293.1 mL/min at one-year) suggesting decreased energetic efficiency. Balance of pulmonary ventilation and perfusion measures reflected altered diastolic filling and could be an early indication of cardiac remodeling (delta VE/VCO2 33.6±5.8 at baseline and 31.8±5.0 at one-year).
- In FA, mean NT-proBNP and Galectin-3 were normal at both visits (74.7±200 and 62±135 ng/L; and 12.2±2 and 12.5±2.62 ng/mL). Troponin T was elevated at both visits (26.3±27.7 and 19.5±15.5 ng/L). These parameters were stable over one year.
- Only CCFS (quantitative evaluation of cerebellar function reflecting upper limb ataxia), not SARA, significantly worsened over one year in FA (p=0.003).

Conclusions

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

The Upper Limb Cardiopulmonary Exercise Test in Friedrich Ataxia Patients

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

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

Dr. CHIARA PANE [1], Dr. Andrea Salzano [1], Dr. Claudia Del Prete [1], Prof. Giuseppe De Michele [1], Prof. Alessandro Filla [1], Prof. Antonio Cittadini [1], Prof. Francesco Saccà [1]

1. *University "Federico II"*

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

Methods:

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

Respiratory gas exchange measurements were obtained breath by breath by a commercially available system (Vmax 29C; Sensormedics, Yorba Linda, CA, USA). Peak oxygen uptake (VO_2max) was recorded at the mean value of VO_2during the last 20 sec of the test. The ventilatory anaerobic threshold was detected by the use of the V-slope method. The ventilation per minute (VE) vs carbon dioxide production (VCO_2) relationship (ventilatory efficiency) was measured by plotting ventilation against VCO_2obtained every 10 sec of exercise (VE/VCO_2slope). The VE/VCO_2slope was calculated as a linear regression function, excluding the non-linear part of the relationship after onset of acidotic drive to ventilation.

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

Results: We studied 54 FRDA patients and 22 healthy controls (HC). Age (35.3±13.8 vs 33.4±9.9; p=0.559), gender (M:F 28:27 FRDA vs 8:14 HC; p=0.248), and BMI (23.1±4.6vs 23.0±3.4; p=0.928) did not differ between groups. VO_2max showed a significant 46% reduction in FRDA patients compared to HC performance (13.95±4.7 for FRDA and 25.68±5.9 mL/Kg/minfor HC; p<0.001; Figure 1). Peak workload (43.87±12.0 for FRDA and 76.18±20.0 watts for HC; p<0.001), and test duration (4.29±2.4 for FRDA and 10.93±2.5 minutes for HC; p<0.001) were both significantly reduced in FRDA patients. Sixty-four percent of FRDA patients reached the anaerobic threshold, as opposed to 32% of HC (p=0.011). VE/VCO_2slope was higher in FRDA as compared to HC (32.87±5.1 for FRDA and 28.48±5.9 for HC; p=0.002).

In FRDA patients, VO_2max inversely correlated with BMI (R=-0.333; p=0.014), interventricular septum thickness (R= -0.281; p=0.039), SARA score (R= -0.434; p=0.001; Figure 2), disease duration (R= -0.397; p=0.003), and 9HPT performance (R= -0.437; p=0.001). VO_2max directly correlated with ADL (R= 0.536; p<0.001), and IADL (R= 0.385;

p=0.004).

Conclusions:FRDA patients showed almost half of the HC's oxygen consumption at the CPX with reduced maximum effort, and reached the anaerobic threshold two times more frequently than HC. This indicates that FRDA patients have a reduced aerobic metabolism that parallels a reduced physical performance. VO_2max correlated with several disease measures, indicating that it may reflect the severity of the disease. Future longitudinal data are needed to describe the CPX performance during disease progression.

Clinical, imaging, biomarker, and molecular delineation of COQ8A-ataxia: a multicenter study of 59 patients

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 27

Saturday, 16th November - 12:15: Clinical Trials and Clinical Trial Design - Oral Abstracts - Abstract ID: 27

Dr. Andreas Traschütz [1], ***Dr. Lucia Laugwitz*** [2], ***Prof. Mathieu Anheim*** [3], ***Dr. Jonathan Baets*** [4], ***Prof. Nazli Basak*** [5], ***Prof. Alexandra Durr*** [6], ***Prof. Rita Horvath*** [7], ***Prof. Thomas Klopstock*** [8], ***Dr. Renato Munhoz*** [9], ***Prof. Filippo M Santorelli*** [10], ***Prof. Bart van de Warrenburg*** [11], ***Prof. Helene Puccio*** [12], ***Dr. Felix Distelmaier*** [13], ***Dr. Ginevra Zanni*** [14], ***Prof. Dave Pagliarini*** [15], ***Prof. Matthis Synofzik*** [16]

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Recent next-generation sequencing techniques have allowed unravelling a large number of novel autosomal recessive ataxia (ARCA) genes. This genetic progress now needs to be translated into preparing first stringent treatment trials. ARCA due to mutations in*COQ8A*(*ADCK3*) might be an especially promising candidate disease, as it might be particularly susceptible to drug treatment, e.g. by coenzyme Q10 (CoQ10) compounds. To pave the way towards trial-readiness, we here exploited a systematic worldwide, multicenter study of 64 patients with biallelic *COQ8A*variants to map the phenotypic, molecular and neuroimaging spectrum of the disease (including in silico modelling and molecular analysis of COQ8A variants, DTI imaging, muscle histology, CoQ metabolites in fibroblasts and blood, and MRI biomarker candidates) and to provide first progression data including response to CoQ10 treatment.

Sixty-four individuals (44 never reported before) from 51 families were included. Structural 3D protein modelling of all 48 different COQ8A variants identified (including 18 novel variants), plus molecular analysis of selected purified variants, demonstrated a wide range of detrimental effects on COQ8 protein functioning.

COQ8A-ataxia presents as a variable multisystemic disease with early-onset cerebellar ataxia (median onset: 7 years, IQR 4-15) as a common denominator (100%, thereof 68% with ataxia at disease onset), yet with frequent additional complicating features, ranging from epilepsy (32%) and cognitive impairment (49%) to exercise intolerance (25%) and hyperkinetic movement disorders (41%), including dystonia and myoclonus even as main presenting symptoms. Missense variants were more frequently associated with multisystemic damage than loss-of-function variants (82-93% vs. 53%, p = 0.029), suggesting gain of function mechanisms. Among missense variants, those affecting helix GQα1 (KxGQ domain) manifested more frequently with developmental delay (50% vs. 16%), epilepsy

(80% vs. 28%) and pyramidal signs (40% vs. 0%, all $p \leq 0.040$). Routine MRI suggests cerebellar atrophy as a universal feature in 100% of the patients, with concomitant cerebral atrophy up to 28%. In-depth DTI specifies that volume loss affects in particular the peripheral anterior and posterior lobe of the cerebellum and diffusely the cerebral cortex. Routine MRI imaging also allowed detecting remarkable infratentorial T2 hyperintensities of the dentate nuclei and the dorsal pons in 28%.

CoQ10 deficiency was found in 73% of muscle biopsies, highlighting that CoQ10 deficiency is a central contributor to disease in this tissue. In contrast, neither CoQ10 nor its CoQ metabolites were deficient in fibroblasts, yet increased reactive oxygen species (ROS) were observed in galactose medium in patients with low CoQ10 levels, indicating an underlying OXPHOS impairment also in this tissue in at least some COQ8A patients.

Cross-sectional (n = 34) and longitudinal (n = 7) assessments of ataxia severity consistently indicated a mild to moderate progression rate (SARA: 0.45/year, 95%CI [0.12 0.77]). Treatment with CoQ10 led to an improvement by anecdotal report in 14/30 patients, and in 8/11 with quantitative longitudinal assessments (SARA: -0.88/year, 95%CI [-1.95 0.19]). Sample size calculations indicate that ≥37 patients per arm are required to demonstrate efficacy for interventions that reduce progression by 50%, and ≥11 patients to demonstrate efficacy of CoQ10 given the outcome variability observed here.

The comprehensive characterization of a large cohort of patients with this extremely rare condition facilitates genetic counselling and patient management, and guides the design of large-scale natural history studies, and upcoming treatment trials in COQ8A-ataxia.

Acknowledgements:

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

Topic: Neurophysiology and the Cerebellum

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

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

Dr. Louise Crowe [1], ***Dr. Louise Corben*** [2], ***Prof. Martin Delatycki*** [2]

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

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

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

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

The influence of HSP subtype on clinical phenotypes in spinocerebellar ataxia type 3

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 276

Dr. Hao-Ling Xu [1], Dr. Arif Sikandar [1], Dr. Min-Ting Lin [1], Dr. Wang Ning [1], Dr. Wan-Jin Chen [1], Dr. Shi-Rui Gan [1]

1. *Department of Neurology and Institute of Neurology, The First Affiliated Hospital of Fujian Medical University, Fujian Key Laboratory of Molecular Neurology*

Background: Spinocerebellar ataxia type 3 (SCA3) is the most common subtype of SCAs worldwide. SCA3 shows remarked clinical heterogeneity. There were five clinical subtypes in SCA, and the type V was termed as hereditary spastic paraplegia (HSP) subtype. Since there were only a few cases have been reported, the existence of HSP subtype is still controversial. The present study intended to analyze the frequency of HSP subtype, the risky factors related to HSP subtype, and explore the influence of the HSP subtype on the clinical phenotypes of SCA3.

Methods:A total of 190 molecular-confirmed SCA3 patients were divided into two groups (HSP subtype and non-HSP subtype), according to whether they had the main signs related to HSP (increased muscle tone, decreased muscle strength, hyperreflexia, positive pathological signs, all are restricted to the lower limbs bilaterally). HSP subtype was further divided into two groups of probable HSP subtype and possible HSP subtype, according to numbers of main signs. Multivariable linear regression was carried out to investigate risky factors of the HSP subtype, and the influence of HSP subtype (HSP subtype vs. non-HSP subtype, and probable HSP subtype vs. possible HSP subtype) on age of onset (AAO), ataxic severity, and ataxic progression.

Results: In all 190 subjects, 45 patients (23.7%) belonged to HSP subtype, of whom 21 (46.7%) were probable HSP subtype and 24 (53.3%) were possible HSP subtype. We found that expanded CAG repeats of *ATXN3* ($p = 0.001$) had a positive influence on HSP subtype. Additionally, patients with HSP subtype had earlier AAO ($p = 0.030$) and severer ataxia ($p = 0.001$), compared to non HSP subtype. Further comparison revealed that the probable HSP subtype had severer ataxia ($p = 0.032$) and faster ataxic progression ($p = 0.022$) than the possible HSP subtype.

Conclusion: The findings of high frequency of HSP subtype, the close connection of CAG repeats in *ATXN3* and HSP subtypes, and the influence of HSP subtype on the clinical phenotypes demonstrated the existence of HSP subtype in SCA3.

Key words

Machado-Joseph Disease (MJD), clinical phenotypes, HSP subtype

A prospective, randomized, controlled trial for the efficacy of repetitive transcranial magnetic stimulation in Machado-Joseph disease

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 275

Dr. Hao-Ling Xu [1], ***Dr. Arif Sikandar*** [1], ***Dr. Min-Ting Lin*** [1], ***Dr. Wang Ning*** [1], ***Dr. Wan-Jin Chen*** [1], ***Dr. Shi-Rui Gan*** [1]

1. *Department of Neurology and Institute of Neurology, The First Affiliated Hospital of Fujian Medical University, Fujian Key Laboratory of Molecular Neurology*

Background: Machado-Joseph disease (MJD), also known as spinocerebellar ataxia type (SCA3), is the most common autosomal dominant ataxia in the world. No effective treatment is currently available for MJD. Repetitive transcranial Magnetic Stimulation (rTMS) is a noninvasive form of brain stimulation, which has been demonstrated to improve symptoms in patients with neurodegenerative cerebellar ataxias. The present study aimed to explore the efficacy of cerebellar rTMS in patients with MJD.

Methods: We recruited 44 molecularly confirmed MJD patients. They were randomly divided into true stimulation group (n=22) and sham stimulation group (n=22). Each of the two groups underwent rTMS stimulation, in which the stimulus coil was placed tangentially (active stimulation) or vertically (sham stimulation), at 1Hz of 1800 pulse with 30 minutes (15 minutes in right lateral and 15 minutes in left lateral, 4 cm from the inion, respectively) for consecutive 15 days. Each patient underwent a pre- and post- clinical evaluation in two groups. We used International Cooperative Ataxia Rating Scale (ICARS) and Scale for the Assessment and Rating of Ataxia (SARA) to evaluate the severity of ataxia, and the Berg balance scale (BBS) to assess the ability to maintain balance. The independent sample T-test was utilized to compare and analyze the changes in all the scores at baseline (d = 0) and at the end (d = 15) in the two groups.

Results: The demographic features of two groups (active stimulation Vs. sham stimulation) showed that the differences between pre- and post-treatment in ICARS, SARA, and BBS were 5.13±2.81 vs 2.68±2.12, 1.80±1.20 vs 0.70±0.88, 4.04±3.46 vs 0.81±2.81, respectively. Further two independent sample T-test analysis found that all the differences of every two pairs showed statistically significant, which illustrated that the patients in the group of active stimulation were significantly improved in the symptoms of ataxia (ICARS: p= 0.02; SARA: p = 0.01) and balance ability (BBS: p = 0.01), compared to the group of the sham stimulation.

Conclusion:A treatment with cerebellar rTMS improved symptoms in MJD patients. rTMS over the cerebellum might represent a promising tool for future rehabilitative approach in patients with MJD.

PROGRESSION AND SIZE EFFECT OF STRUCTURAL CHANGES IN SCA3/MJD: A LONGITUDINAL STUDY OF MULTIMODAL NEUROIMAGING

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 264

Ms. Camila Piccinin [1], ***Mr. Pedro Moyses*** [2], ***Mr. Jean Levi Paiva*** [3], ***Dr. Alberto Martinez*** [1], ***Dr. Thiago Rezende*** [1], ***Dr. Marcondes Cavalcante França Jr.*** [1]

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Background/ Objectives: Spinocerebellar ataxia type 3 or Machado Joseph disease (SCA3/MJD) is the most prevalent autossomal dominant ataxia described. The CAG repeat expanded mutation in the coding region of ATXN3 is the underlying cause leading to a variable phenotype mostly characterized by ataxia, peripheral neuropathy, dystonia and or rigidity and ophtalmoplegia together with non-motor symptoms. Such complex disorder has arisen an increasing interest for establishing a profile of the anatomical progression of the disease. Rezende and colleagues performed a neuroimaging cross-sectional study of 91 patients including 12 non-ataxic mutation carriers and found abnormalities from the spinal cord, cerebellar peduncles and substancia nigra extending later in the disease course to cerebral cortex and subcortical structures in a caudal-rostral manner. Some of the changes were detect even in the presintomaptic group supporting the idea that neuroimaging may be more sensitive than clinical scales. The first longitudinal study analyzed 51 SCA3/MJD in a 12.5 months follow-up but it was not possible to find any significant progression, probably due to short duration. A multicenter European project addressed this issue by evaluating the gray matter of 19 SCA3/MJD patients in a 2-year follow-up. The most sensitive to change structure was the volume of the striatum which was also more sensitive than clinical scales. We here built a 5-year follow-up of 23 SCA3/MJD patients using a wide mutimodal neuroimaging approach. For all significant positive results, we calculated the rate of progression aiming to find the most sensitive to change parameter amongst all methods including the SARA scale for further comparison.

Methods: Twenty-three patients with SCA3/MJD and 22 healthy controls underwent 3T MRI and clinical evaluations in two time-points (mean time interval between patient acquisitions: 5.0 years and controls: 4.6 years). We used SpineSeg software for cervical spinal cord evaluation, ENIGMA CERES/ SUIT protocol for cerebellar analysis, Multiatlas for deep gray matter and DTI-Multiatlas for white matter integrity evaluation. For the statistical analyses we used the absolute numbers of the results in each region analyzed to calculate the amount of change, so we basically subtracted the measures obtained in the first MRI (T1) from the second MRI (T2) for each patient and each control. Then we used the result found to access group differences (patients vs controls) by performing an ANOVA test. All analyzes were covariated for age and sex, and Bonferroni correction was applied (adjusted p-value <.05). The “effect sizes” were calculated for each statistically significant result including the SARA scale.

Results: Multiatlas assessment found significant changes in left putamen, left thalamus and right medulla being the greater effect size in the left thalamus (0.78) (figure 1). Multitlas-DTI was performed in 21 SCA3/MJD patients and 22 controls and significant increase of radial diffusivity were reported bilaterally in the corticospinal tracts, in the left superior and right inferior cerebellar peduncles which had the greatest effect size of all analyses (1.29) (figure 2). For measures of mean diffusivity, we found significant changes in the same areas but the left corticospinal tract. We found large effect sizes bilaterally in corticospinal tracts, left superior and right inferior cerebellar peduncles. Although both Spineseg and Ceres-Suit analyses have shown reduced volumes in cerebellum and spinal cord throughout follow-up, it was not significant when compared with controls. The mean difference in SARA scale

comparing the two time points was 3.5 points (ranging from -3.5 – 12), which gives a ratio of 0.68/year and effect size of 0.81.

Discussion/ Conclusions: Our study demonstrated that cerebellar peduncles and corticospinal tracts are the areas most susceptible to longitudinal structural changes in SCA3/MJD. Interestingly, the progression of the right inferior cerebellar peduncle followed by the middle peduncles confer a measure more sensitive than the SARA scale, confirming previous studies but addressing the role of the white matter bundles. Regarding the cerebellum and spinal cord, despite alterations in these structures have been consistently reported, they are affected very early in the disease course and were probably already abnormal in the baseline image thus a floor effect may explain the non-significative results. Since SCA3/MJD is a neurodegenerative and dynamic disorder, establishing a profile of anatomical progression as our study did is essential to define the best targets for treatment but also to determine potential structural markers of disease modification for clinical trials.

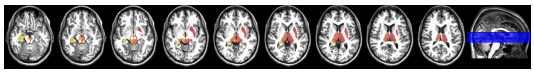

Image1.png

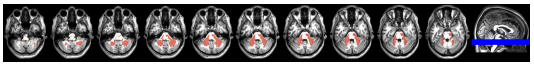

Image2.png

Patients with cerebellar ataxia reporting gaze instability demonstrate abnormal oculomotor patterns during passive head rotation

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 68

Ms. Jennifer Millar [1]***, Dr. Michael Schubert*** [2]

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Author: Jennifer L Millar, MS, PT, Johns Hopkins Medical Institutions

Purpose: To describe eye and head movement patterns in patients diagnosed with cerebellar ataxia and, as well as to begin to address the question, “Are gaze stability exercises effective in reducing symptoms of oscillopsia in cerebellar ataxia patients?”

Background: Patients, diagnosed with cerebellar dysfunction, referred for physical therapy commonly report complaints of gaze instability (perception of blurred vision with head movement). There is scant literature available describing passive eye and head movement patterns in cerebellar ataxia patients. In ataxia patients who have reported gaze instability, we have started to include video head impulse testing (HIMP) as part of their clinical PT evaluation as well as utilize a recently developed oscillopsia measure, validated in the vestibular population1.

Methods: 22 patients with cerebellar dysfunction (diagnosed with spinocerebellar ataxia types 2, 7, 8, or cerebellar ataxia of unknown etiology with family and without family history), completed the Oscillopsia Functional Index Scale (OFIS)1. The HIMP protocol in the yaw and vertical planes and the Suppression Head Impulse Test (SHIMP) in the yaw plane was conducted in 22/22 and 3/22patients respectively. Additional clinical oculomotor tests and functional outcome measures were recorded.

Results: Varied eye movement patterns were observed with HIMP and SHIMP testing including 1) anti-compensatory saccades with normal, hypometric, or hypermetric VOR 2) hypermetric VOR 3) hypometric VOR responses without compensatory saccades 4) hypometric VOR responses with overt/covert saccades, 5) hypometric bilateral VOR with saccades. Functional outcome measures revealed self-reported gaze stability and balance confidence and well as functional performance impairments, below normative values. (See table).

Relevance: Our data reveals abnormal oculomotor behavior during head rotation in patients with cerebellar pathology and a report of gaze instability, including an undiagnosed cerebellar ataxia with bilateral vestibular dysfunction - CABV2. Our data suggests rehabilitation to address the gaze instability is warranted, though future study on efficacy of such training is unknown.

References

1 Anson ER, Gimmon Y, Kiemel T, Jeka JJ, Carey JP, A Tool to Quantify the Functional Impact of Oscillopsia. Frontiers in Neurology 2018; 9: 142

Table 1: Functional Outcome Measures

	OFIS	ABC (%)	10 MWT (m/s)	TUG (seconds)	TUG Cog (seconds)	DGI	SARA
Patient mean ± 1 SD	72.2 ±33.4	51.5 ±19.5	1.0 ± 0.3	12.8 ± 7.3	17.9 ± 8.5	12.1 ± 6.6	14.1 ± 4.7
Normative values	< 12 *	> 80%	>1.3 m/s	<11	< 15	> 22/24	< 0.4 **

Table 1 functional outcomes.png

Topic: Mechanism of Disease

Interruptions as disease modifiers and repeat regulators

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

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Background

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

Materials and methods

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

Results

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

Conclusions

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

disease severity.

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

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

Novel polyserine antibody as a common tool to identify RAN positive disorders: results from SCA2 and SCA3 cerebellum

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RAN translation has now been reported in nine different microsatellite expansion disorders caused by non-coding as well as coding repeat expansion mutations, including Huntington's disease. A major limitation determining if RAN translation contributes to the pathogenesis of other expansion diseases is the lack of available tools to detect RAN proteins. Antibodies against homopolymeric proteins are challenging to develop, which makes the role of RAN translation difficult to test for CAG expansion disorders. To explore the contribution of RAN translation across CAG•CTG expansion disorders, we generated an antibody against polySerine repeats. The specificity of this new repeat antibody was demonstrated in transfected cells using both Western blot and immunofluorescence approaches. Additionally, its suitability as an efficient immunostaining tool to detect RAN proteins in human autopsy samples has been validated in previously characterized RAN positive disorders, including HD and SCA8. This novel polySerine repeat antibody is an efficient tool to explore RAN protein accumulation across CAG/CTG expansion diseases, including those not yet characterized for RAN.

Using this new validated tool we have performed immunostaining in SCA2 and SCA3 postmortem brain tissue. Here we show that RAN polySerine proteins accumulate in the cerebellum of SCA2 and SCA3 patients, either as nuclear staining or neuropil microaggregates. polySerine prominent staining is found in Bergman glia, cortical white matter regions and deep white matter regions of the cerebellum. Addtionally, RAN positive regions show alterations in axonal myelination and neuroinflammatory changes in the absence of significant polyGln signal.

Understanding the role of polySerine accumulation in SCA2 and SCA3 will provide novel insight into the mechanisms of disease and novel therapeutic targets.

Reversing increased levels of O-GlcNAc transferase in Machado-Joseph disease improves the molecular phenotype via autophagy activation

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Ms. Priscila Pereira Sena [1], ***Dr. Jonasz Jeremiasz Weber*** [2], ***Dr. Jana Schmidt*** [3], ***Dr. Jeannette Hübener-Schmid*** [2], ***Prof. Olaf Riess*** [3], ***Dr. Thorsten Schmidt*** [3]

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Dysregulation of brain glucose uptake has been linked to diverse neurodegenerative conditions, including Amyotrophic lateral sclerosis, Alzheimer's, Parkinson's and Huntington's diseases. The monosaccharide *O*-linked N-acetylglucosamine (*O*-GlcNAc) is a derivative of glucose metabolism and can be attached to a target protein by the enzyme *O*-GlcNAc transferase (OGT). This process, known as*O*-GlcNAcylation, is a highly dynamic and abundant posttranslational modification that represents a potential link between a dysfunctional neuronal glucose uptake and neurodegeneration, since several neuronal key proteins have been shown to be *O*-GlcNAcylated. Despite this fact, little is known about *O*-GlcNAcylation in the context of polyglutamine disorders.

Our work aimed to evaluate this posttranslational modification in Machado-Joseph disease (MJD), the most common autosomal dominant ataxia. MJD is caused by an elongated polyglutamine stretch within the deubiquitinating enzyme ataxin-3, leading to an increased propensity for aggregation. It was observed that MJD patients present cerebral glucose hypometabolism, and a cohort of early onset patients showed higher insulin sensitivity. A recent work proposed that moderate caloric restriction improves motor coordination in an MJD model via sirtuin-1, a NAD-dependent deacetylase whose activity and levels are known to be modulated by *O*-GlcNAcylation. Moreover, *O*-GlcNAcylation impacts autophagy, a dysregulated pathway in fibroblasts and postmortem brains of MJD patients. We analyzed global *O*-GlcNAc and OGT levels in cells expressing polyglutamine expanded ataxin-3, as well as immortalized patient-derived fibroblasts and brain samples from a transgenic MJD mouse model. Cellular and animal ataxin-3 knockout models were also tested for the possible influence of wild-type ataxin-3 on *O*-GlcNAc and OGT. Our results demonstrate that global *O*-GlcNAc and baseline OGT levels are elevated in MJD cellular and animal models. Interestingly, ataxin-3 influences OGT degradation, yet contributing to the control of autophagy. Furthermore, genetic and pharmacological OGT inhibition promotes autophagy activation, as well as degradation of soluble and aggregated forms of polyglutamine expanded ataxin-3. Our study sheds new light on a yet unknown physiological function of ataxin-3 and may contribute to the development of therapeutic approaches for MJD and other yet incurable neurodegenerative conditions, via targeting the glucose uptake-related *O*-GlcNAc pathway.

Calpain-1 ablation ameliorates molecular disease hallmarks in cell and mouse models of Machado-Joseph disease

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 235

Dr. Jonasz Jeremiasz Weber [1], ***Dr. Stefan Hauser*** [2], ***Prof. Athar Chishti*** [3], ***Prof. Olaf Riess*** [1], ***Dr. Jeannette Hübener-Schmid*** [1]

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Proteolytic fragmentation of polyglutamine-expanded ataxin-3 is a concomitant and modifier of the molecular pathogenesis of Machado-Joseph disease (MJD), the most common autosomal dominant cerebellar ataxia. Calpains, a group of calcium-dependent cysteine proteases are important mediators of ataxin-3 cleavage. Pharmacologic and genetic approaches lowering calpain activity showed beneficial effects on molecular and behavioural disease characteristics in MJD model organisms. However, specifically targeting one of the calpain isoforms by genetic means has not yet been investigated as a potential therapeutic approach. In our study, we first tested if calpains are overactivated in the MJD context at baseline. Secondly, we evaluated if calpain-1 ablation in cell and animal models of MJD yields in disease phenotype-ameliorating effects. For this, calpain activation was measured in patient-derived fibroblasts and polyQ-expanded ataxin-3-transfected cell lines. Calpain-1 expression was lowered in ataxin-3-transfected cells by an esiRNA-based approach and abolished *in vivo* by crossbreeding YAC84Q MJD mice with *Capn1* knockout animals. Western blot analyses showed that cleavage of the calpain substrate α-spectrin is elevated in cell models of MJD, and both calpain-1 knockdown in cells as well as *Capn1* knockout in MJD mice led to reduced calpain system activation and polyQ-expanded ataxin-3 cleavage. Ataxin-3 aggregation was not affected in YAC84Q/*Capn1* knockout mice, however, neuropathology markers such as DARPP-32 and synapsin-1 levels were partly rescued. Here, we showed that targeting ataxin-3-cleaving calpains by genetic means directly can counteract an evidenced calpain overactivation in MJD cell and mouse models, decrease ataxin-3 proteolysis and attenuate detrimental effects on neuronal markers *in vivo.* Thus, our results reaffirm calpains as an auspicious therapeutic target for MJD.

Frataxin deficiency causes excessive Drp1-dependent mitochondrial fragmentation

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Introduction:

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

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

Methods:

Human fibroblasts derived from healthy individuals or FRDA patients were cultured and transfected following standard protocols. P110 and a scrambled form of this peptide were obtained from Genscript and reconstituted in sterile ddH_2O. Fibroblasts were incubated with 1μM P110 for 72 hours before staining and immunofluorescence imaging. The cells were fixed to glass coverslips, probed for proteins of interest, incubated with fluorophore-conjugated secondary antibodies, and imaged using a Leica confocal microscope and LasX software.

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

Results:

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

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

Discussion:

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

Conclusions:

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

Serotonergic signaling activation suppresses proteotoxicity

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The failure of proteins to fold or to maintain proper conformation may result in the loss of essential functions and in the formation of toxic aggregates. Therefore, quality control and the maintenance of protein homeostasis (proteostasis) are critical for cell and organism health. Deficiencies in proteostasis are associated with many diseases, including spinocerebellar ataxias. Previously, we found that modulation of serotonergic signaling by pharmacological or genetic inhibition of the serotonin (5-HT) transporter suppressed mutant ataxin-3 aggregation, the protein involved in Spinocerebellar ataxia type 3 (SCA3) or Machado-Joseph disease (MJD).

Here, we propose serotonergic signaling as a biological proteostasis regulator and investigate the role of serotonergic pathways on the maintenance of protein folding and function, regardless of the identity of the protein. We used proteostasis sensors to measure the activity status of the proteostasis network, and unbiased genome-wide transcriptomic analyses to identify novel pathways.

By combining compound treatment with genetic tools, we demonstrate that modulation of 5-HT signaling by citalopram (CIT) and other Selective Serotonin Reuptake Inhibitors (SSRIs) suppresses Tau-induced neurotoxicity in *C. elegans*. CIT treatment is also able to prevent loss of dopaminergic neurons, toxin-induced and alpha-synuclein-mediated, in Parkinson's disease mimicking *C. elegans* models. Previous studies in *C. elegans*have used temperature-sensitive neuron-specific mutant proteins as folding sensors. These mutations manifest a motor phenotype at a restrictive temperature, or at permissive temperature upon co-expression with aggregating polyQ proteins or during normal aging. Strikingly, serotonergic signaling activation with CIT suppresses their toxic phenotype at what would normally be restrictive temperatures. Next, we used as a folding sensor an exogenous protein that has no biological function in *C. elegans* cells and is known to be chaperone-dependent for folding and refolding: a conformationally destabilized variant of the firefly luciferase protein. Our preliminary findings suggest that 5-HT signaling activation improves luciferase folding and re-folding upon heat stress. RNA sequencing of SCA3 mice brains treated with CIT highlighted some pathways that may underlie 5-HT-mediated suppression of proteotoxicity, and which we are currently characterizing further.

Our findings suggest 5-HT signaling modulation as a disease-modifying strategy for proteinopathies, the mechanism of which we are contributing to illuminate.

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

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

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

The ataxia protein sacsin impacts on focal adhesion dynamics and cell migration

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Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS) is a childhood-onset neurological disease with pyramidal spasticity and cerebellar ataxia. ARSACS results from mutations in the *SACS*gene that encodes sacsin. Previously we identified that loss of sacsin results in reduced mitochondrial health and altered organization of the intermediate filament cytoskeleton.

Sacsin is a modular protein with conserved domains that suggest a proteostasis associated function. We hypothesized that loss of sacsin would alter solubility of its client. Using proteomics approaches, we identified sacsin interactors that accumulated in an insoluble fraction from sacsin knockout cells. These included cytoskeletal and focal adhesion associated proteins. Putative sacsin interactors with altered solubility included vimentin and neurofilament, which have previously been identified as having abnormal organisation and bundling in sacsin knockout cells. Further intermediate filament proteins, including glial fibrillary acidic protein, desmin and peripherin, where identified by our mass spectrometry analysis, as were alpha tubulin isoforms and alpha actinin proteins. These data suggest a wider impact of loss of sacsin on intermediate filaments and cytoskeleton more generally.

Alpha actinin proteins are actin cross linking proteins that play an important role in formation and maturation of cell adhesions. Confocal microscopy revealed that the number and structure of focal adhesions were altered in sacsin knockout cells (generated by CRISPR/Cas 9 genome editing). Moreover, fluorescence recovery after photobleaching demonstrated that focal adhesion dynamics were reduced and directional migration impaired in sacsin knockout cells.

To understand mechanisms underlying the altered focal adhesion dynamics we investigated activation of the focal adhesion kinase signaling pathway. This showed a reduction in phosphorylation of Focal Adhesion Kinase (FAK), c-Jun N-terminal kinases (JNK) and Paxillin. We also observed that levels of Phosphatase and Tensin homolog (PTEN), which is a negative regulator of FAK signalling, were increased in sacsin null cells. We therefore chose to investigate the specific effect of reducing the expression of PTEN, as a way to increase pFAK levels in sacsin knockout cells. Sacsin knockout cells transfected with small interfering RNA (siRNA) targeting PTEN exhibited elevated pFAK levels that corresponded with increased incidence of focal adhesions. Together these data suggest that altered PTEN/FAK signalling contributes to the sacsin knockout cell adhesion and migration phenotypes.

PTEN has a critical role in the regulation of neuronal development, axonal growth, and synaptic plasticity. Importantly, in addition to neurodegeneration, ARSACS is thought to have a neurodevelopmental component. It thus seems possible that altered PTEN levels in sacsin deficient cells could impact on neurodevelopment in ARSACS patients.

Epigenetic and enhancer alterations compromise the maintenance of neuronal identity in spinocerebellar ataxia type 7

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 129

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Spinocerebellar Ataxia Type 7 (SCA7) belonging to ADCAs is pathomechanistically related to a group of polyglutamine expansion disorders, which include SCA 1-3, 6 and 17, and Huntington's disease. SCA7 affects primarily brainstem, retina and cerebellum. The distinctive feature of SCA7 among ADCAs is a progressive visual impairment due to a cone-rod photoreceptor dystrophy. Ultrastructural analysis of SCA7 mouse models revealed that photoreceptors progressively lose their outer segments, a structure essential for phototransduction, which requires daily renewal. The lack of outer segment renewal correlates with decreased expression of photoreceptor-specific genes, suggesting that progressive breakdown of photoreceptor cell identity accounts for SCA7 retinopathy.

SCA7 is due to a polyglutamine expansion in ATXN7, a component of the multiprotein Spt-Ada-Gcn5 Acetyltransferase (SAGA) complex, a highly conserved coactivator of transcription. SAGA harbors two chromatin remodeling activities: acetylation of histone 3 on lysine 9 (H3K9ac) on gene promoters involved in transcriptional initiation, and deubiquitination of histone 2B (H2Bub) on gene bodies crucial for transcription elongation.

The role of SAGA in SCA7 pathogenesis and the tissue specific genetic breakdown remains to be elucidated. We addressed this issue using our new SCA7$^{140Q/5Q}$ KI mouse model, which recapitulates cardinal features of SCA7. We found that the global level of H3K9ac is strongly decreased in the retina and cerebellum of SCA7 mice. Using ChIP-seq approach, we confirmed the general decrease of H3K9ac in most gene promoters in SCA7 retina, which alone cannot explain the specific gene deregulation. Interestingly, we discovered that photoreceptor identity genes, which are strongly downregulated in SCA7, harbor an unusual broad profile of H3K9ac that encompasses the entire gene. We further show that H3K9ac broad profiles at photoreceptor gene loci coincide with the presence of a broad H3K27ac, a signature of active enhancers. Furthermore, using vicinity criteria we identified enhancer RNAs (eRNAs), annotated to the photoreceptor identity genes possessing an atypical H3K9ac/H3K27ac enhancer signature. Finally, we found a synchronized expression of eRNAs and photoreceptor identity genes during retinal development, suggesting a regulatory role of these eRNAs. In SCA7 symptomatic mice, hypoacetylation of the loci containing H3K9ac/H3K27ac broad profiles and decreased eRNA levels correlate with the downregulation of photoreceptor identity genes.

Thus, our results support the view that epigenetic and enhancer alterations compromise the maintenance of neuronal identity in SCA7.

The importance of the ATXN3 haplotype status for the pathophysiology of MJD/SCA3

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 168

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The *ATXN3* gene, affected in Machado-Joseph disease (MJD) or Spinocerebellar Ataxia type 3 (SCA3), contains three nonsynonymous single nucleotide polymorphisms within its coding region which cause amino acid changes or even a premature stop in the encoded ataxin-3 protein: The polymorphisms $A^{669}TG/G^{669}TG$ (in exon 8), $C^{987}GG/G^{987}GG$, and TAA^{1118}/TAC^{1118} (both in exon 10). In MJD/SCA3 patients, two major haplotypes of these polymorphisms can be found: the so called Flores haplotype (ACA) and the São Miguel haplotype (GGC), named after Azorean Islands (Gaspar et al. Am J Hum Genet 2001). Therefore, depending on the haplotype, MJD/SCA3 patients express different versions of ataxin-3. Here we analyzed the consequences of these haplotypes on pathophysiological mechanisms in MJD/SCA3.

We examined the significance of ataxin-3 isoforms generated by alternative splicing of the *ATXN3* gene and the effect of polymorphic amino acid changes and the premature stop on major aspects of the physiological function of ataxin-3 as well as their impact on main disease mechanisms. We further determined the frequency of each haplotype in a cohort of European MJD/SCA3 patients.

At the physiological level, we show that alternative splicing and the premature stop codon alter ataxin-3 stability and that ataxin-3 isoforms differ in their enzymatic deubiquitination activity, subcellular distribution, and interaction with other proteins. At the pathological level, we found that the expansion of the polyglutamine repeat leads to a stabilization of ataxin-3 and that ataxin-3 isoforms differ in their aggregation properties. The stop codon, present in the Flores haplotype (ACA), aggravates pathology. Interestingly, we observed functional interactions between normal and polyglutamine-expanded *ATXN3*allelic variants which modify the physiological and pathophysiological properties of ataxin-3. Our findings indicate that the haplotype, alternative splicing, and interactions between different ataxin-3 isoforms affect not only major aspects of ataxin-3 function but also important pathophysiological mechanisms of MJD/SCA3. The *ATXN3*haplotype status (both of the normal and the expanded allele) should be considered as important disease modifier as well as in future clinical trials involving MJD/SCA3 patients.

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

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

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

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

The second issue concerns the replication/transcription conflict at the expanded GAA-repeat, which could drive further expansion and somatic instability of the repeat[1-3]. However, the mechanisms involved in GAA-trinucleotide expansion in FRDA families are far from being completely understood[6-7]. To understand whether *FXN*transcription may be modulated along the cell cycle, lymphoblastoid cells derived from FRDA patients or healthy relatives were FACS-sorted in subfractions of different cell cycle stages. We applied RNA-FISH with multiple pre-mRNA probes (Stellaris®) to monitor RNA initiation/elongation at *FXN*. By applying such approach we are deepening the possible involvement of replication/transcription conflicts in GAA-repeat expansion.

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

pansion after transcription rescue [6,7].

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Association of a variant in the promoter region of DNAJB6 gene with onset of Machado-Joseph disease in patients from South Brazil

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 190

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Machado-Joseph Disease or spinocerebellar ataxia type 3 (MJD/SCA3) is an autosomal dominant disease, characterized by gait ataxia and loss of other neuromotor functions. MJD/SCA3 is due to an expansion of the CAG repeat on the ATXN3 gene. Length of alleles with an expansion varies from 60 to 80 CAG repeats, while in normal alleles, CAG length varies from 12 to 43 repeats. The age at onset (AO) of symptoms is inversely correlated to repeat length. However, considering that patients can develop the disease earlier or later than expected according to their expanded CAG length, additional genetic and/or ambiental factors may affect AO. Based at previous work, one of the potential AO modulators is DNAJB6. This chaperone prevents intranuclear aggregation when in presence of a mutant ataxin-3 as well as in other similar mutant proteins. Thus, the aim of this study was to evaluate the effect of variations in the promoter region of the DNAJB6 gene on AO of MJD/SCA3 patients. Sample population was composed by patients from Rio Grande do Sul, being 19 with early AO and 26 with late AO, as well as 20 controls. Genomic DNA was isolated and CAG repeat length was assessed by PCR. The promoter region of DNAJB6 (1373 bp) was divided into 4 overlapping subregions and sequenced by Sanger, followed by capillary electrophoresis. From 38 previously reported SNPs, only those with frequency higher than 5% in the less common allele were used for statistical analysis, resulting in 4 SNPs. Then a $\chi 2$ test was performed for allele, genotype, and reconstructed haplotypes data. The linkage equilibrium measure dropped, more than expected due to the genomic distance, among the further SNP from the transcription start site (TSS) and the other ones, suggesting a conservation ratio inversely proportional to the transcriptional start distance. Haplotype reconstruction generated two haplotypes with high frequency in our cohort: GGGC (0.55) and CAAT (0.32). Five ploidies presented a haplotype with good fit for late onset group ($p=0.0572$). No significant fit was seen in any group in the $\chi 2$ for alleles. CC genotype in rs3802101 showed a significant correlation ($p=0.0124$) in the late onset patients group. Therefore, we observe an association of this variation with a delay in the AO of MJD/SCA3 patients. The outcome of this study can suggest a direct effect on regulation of transcription due to the location of this SNP 159 bp upstream the transcription start site (Financial support: PIBIC-CNPq, FIPE-HCPA, FAPERGS).

Absence of iron-responsive element-binding protein 2 causes a novel neurodegenerative syndrome

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 41

Dr. Gregory Costain [1], ***Dr. Manik Ghosh*** [2], ***Dr. Nunziata Maio*** [2], ***Ms. Amanda Carnevale*** [3], ***Dr. Yue Si*** [2], ***Dr. Tracey Rouault*** [2], ***Dr. Grace Yoon*** [1]

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Altered distribution of cellular iron may contribute to the pathogenesis of both common and rare neurodegenerative conditions, such as Alzheimer's disease, Parkinson's disease, Friedreich ataxia, Huntington's disease, and the neurodegeneration with brain iron accumulation disorders. In mammals, two iron-regulatory proteins (IRPs) shape the expression of the iron metabolism proteome. Targeted deletion of Ireb2 in a mouse model causes profoundly disordered iron metabolism, leading to functional iron deficiency, anemia, erythropoietic protoporphyria, and a neurodegenerative movement disorder. Using exome sequencing, we identified the first human with bi-allelic loss-of-function variants in the gene IREB2 leading to an absence of IRP2. This 16-year-old male had neurological and haematological features that emulate those of Ireb2 knockout mice, including neurodegeneration and a treatment-resistant choreoathetoid movement disorder. Cellular phenotyping at the RNA and protein level was performed using patient and control lymphoblastoid cell lines, and established experimental assays. Our studies revealed functional iron deficiency, altered post-transcriptional regulation of iron metabolism genes, and mitochondrial dysfunction, as observed in the mouse model. The patient's cellular abnormalities were reversed by lentiviral-mediated restoration of IRP2 expression. These results confirm that IRP2 is essential for regulation of iron metabolism in humans, and reveal a previously unrecognized subclass of neurodegenerative disease with ataxia and movement disorder. Greater understanding of how the IRPs mediate cellular iron distribution may ultimately provide new insights into common and rare neurodegenerative processes, and could result in novel therapies.

Nonneuronal contributions to SCA3 disease pathogenesis.

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 49

Ms. Annie Zalon [1], Mr. Nick Stec [1], Ms. Amanda Pierce [1], Dr. Hongjiu Zhang [1], Dr. Yuanfang Guan [1], Dr. Henry Paulson [1], Dr. Hayley McLoughlin [1]

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Spinocerebellar ataxia type 3 (SCA3), also known as Machado-Joseph Disease (MJD), is the most common dominantly inherited ataxia in the world yet there is no effective treatment for this relentlessly progressive and fatal disease. With the goal of preventive therapy for SCA3, several recently completed or ongoing studies seek to reduce levels of the disease protein, including our recent study assessing the longitudinal efficacy of antisense oligonucleotide (ASO) therapy for SCA3. A necessary next step toward therapeutic success is a thorough assessment of SCA3 molecular changes over time, so that we can better understand potential neuroprotective pathways and identify promising biomarkers of disease. In our study, we compared transgenic SCA3 mice possessing the full-length human mutant *ATXN3* gene to wild type littermates. Through RNA sequencing (RNA-seq), we longitudinally characterized the central nervous system (CNS) transcriptome during early-, mid-, and late-stage of disease in two highly affected brain regions, the cerebellum and brainstem. We uncovered early, and potent gene expression changes related to myelination and cholesterol biosynthesis. To date, research efforts in SCA3 have largely focused on understanding neuronal dysfunction and neuronal cell death, but recent evidence from human and animal studies reveals that glial cells also contribute to SCA3 disease progression. Of glial cell types in the mammalian brain, oligodendrocytes are most abundant and play integral roles in myelin-rich white matter structures that are vulnerable to SCA3 disease. Our ongoing studies in the SCA3 mouse model suggest a delay in oligodendrocyte maturation relative to sex- and age-matched wildtype littermates. Our lab is currently investigating mutant ATXN3 behavior in oligodendrocytes, including whether oligodendrocyte dysfunction in SCA3 is a cell autonomous effect or the byproduct of broader CNS involvement. SCA3, it now seems clear, is not simply a neuronal disease and the search for therapeutic strategies and disease biomarkers for this fatal disorder will need to account for nonneuronal involvement, especially oligodendrocytes.

Friedreich cardiomyopathy is a secondary desminopathy

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

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

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In-vivo identification of Diseases Modifiers in a Drosophila model of Friedreich´s ataxia

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

Prof. Stephan Schneuwly [1], ***Dr. Juan Antonio Navarro Langa*** [1]

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

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

…and it continues providing new insights into the pathology

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

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

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

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

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

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

Microglial and astrocytic pathology in a mouse model of Machado-Joseph disease

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 272

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Machado-Joseph disease (MJD) is a genetically determined neurodegenerative disease of adult onset, caused by expansion of a polyglutamine tract within the protein ataxin-3. Neuropathological analysis of the brain of MJD patients reveals major neuronal loss in the deep cerebellar nuclei, nuclei of the pons, subthalamic and red nuclei, and involvement of the spinocerebellar tracts. Astrogliosis is observed in brains of MJD patient's post-mortem, but it has classically been interpreted as a reaction to neuronal demise. Our goal in this study is to determine the contribution of glial cells for disease initiation and progression in MJD, using a well characterized animal model of the disease, the CMVMJD135. We found a region-specific astrocytic pathology in symptomatic mice (34 weeks of age); whereas in the substantia nigra and spinal cord (affected regions) a classical astrocytic reactivity is present, in the pontine nuclei (affected region) we found a general hypotrophy of the astrocytes. Nevertheless, no differences in the expression levels of the glutamate transporter EEAT2 were detected in the brainstem and spinal cord, suggesting functional astrocytes. These results point to a time- and region-dependent astrocytic pathology. Interestingly, mRNA expression analysis in young symptomatic mice of inflammation-related molecules revealed an up-regulation of anti-inflammatory molecules such as arginase-1 and Il4, suggesting an earlier M2-like phenotype. Moreover, as the disease progresses, we observed a shift to a M1-like/mixed phenotype, with 34 weeks-old animals showing an up-regulation of Tnfa, Il1b, Ccl2, CD86 and Il10 in the brainstem and spinal cord. Intriguingly, the mRNA expression levels of arginase 1 and peroxiredoxin-2, anti-inflammatory-related enzymes, were significantly decreased. Furthermore, flow cytometry showed an increase of classical microglia activation markers, as well as an increase of macrophages in the brain of CMVMJD135 mice at 34 weeks of age, which is in accordance to the increased Ccl2 expression. Altogether, these results support an involvement of astrocytes and microglia in the pathogenesis of MJD.

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

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

Dr. Elena Dedkova [1], ***Ms. Claire B. Montgomery*** [1], ***Mr. Chun Kiu Hui*** [1], ***Dr. Sandipan Datta*** [1], ***Dr. Alexey A. Tomilov*** [1], ***Prof. Gino Cortopassi*** [1]

1. *UC Davis*

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

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

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

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

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

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

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

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

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

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

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

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

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

Dr. Rosella Abeti [1], ***Dr. Alexander Brown*** [2], ***Dr. Marta Maiolino*** [3], ***Prof. Sandip Patel*** [4], ***Prof. Paola Giunti*** [5]

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Background. Friedreich's Ataxia (FRDA) is a neurodegenerative disorder, characterized by degeneration of dorsal root ganglia, cerebellum and cardiomyopathy. Heart failure is one of the most common causes of death for FRDA patients. Deficiency of frataxin, a small mitochondrial protein, is responsible for all clinical and morphological manifestations of FRDA. Our aim was to investigate the unexplored Ca2+ homeostasis in cerebellar granule neurons (CGNs) and in cardiomyocytes of FRDA cellular models to understand the pathogenesis of this condition. Ca2+ homeostasis in neurons and cardiomyocytes is not only crucial for the cellular wellbeing but more importantly to generate action potential in both neurons and cardiomyocytes.

Methods. By challenging Ca2+ homeostasis in CGNs, and in adult and neonatal cardiomyocytes of FRDA models, we have assessed the impact of frataxin decrease on both neuronal and cardiac physiopathology. Using both fluorescence and confocal microscopy we have measured reactive oxygen species (ROS), lipid peroxidation, mitochondrial membrane potential, cytosolic and mitochondrial Ca2+ alterations in FRDA-like cells. We then used a hypoxia-reperfusion injury to induce cell death and then test the effect of Vitamin E (an antioxidant).

Results. Our results showed that oxidative stress is present in our FRDA-like cells and that Ca2+ homeostasis is altered in CGNs and cardiomyocytes. CGNs showed a Ca2+ mishandling under depolarizing conditions and this was also reflected in the endoplasmic reticulum (ER) content. In cardiomyocytes we found that the sarcoplasmic reticulum (SR) Ca2+ content was pathologically reduced, and that mitochondrial Ca2+ uptake was impaired. In cardiomyocytes, our results showed that ryanodine receptors (RyRs) may be leaking and expel more Ca2+ out from the SR. At the same time mitochondrial uptake was altered. Similarly to other neurodegenerative conditions we have witnessed in FRDA the increase of oxidative stress. It is known that oxidative stress modulates key players at the SR/ER and mitochondrial level that usually restore the Ca2+ homeostasis. By using an antioxidant such as Vitamin E these alterations were restored. Moreover, Vitamin E protected from cell death induced by hypoxia-reperfusion injury. Our results revealed novel properties of Vitamin E as potential therapeutic tool for FRDA cardiomyopathy.

Conclusion. Our findings demonstrate that in both neurons and cardiomyocytes the decreased Ca2+ level within the stores has a comparable detrimental impact in their physiology. Moreover, Vitamin E helps protecting cardiomyocytes from the Ca2+ mishandling found in FRDA like models.

Acknowledgements:

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

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

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

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

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

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

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

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

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

1. *Novoheart,* ***2.*** *The Chinese University of Hong Kong,* ***3.*** *Pfizer Inc*

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

Lipid metabolism disruption as a consequence of mitochondrial dysfunction in FA

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

Ms. Elaine Ho [1], Dr. Grazia Cotticelli [2], Dr. Robert Wilson [2], Dr. David Lynch [2], Prof. Ian Blair [3], Dr. Clementina Mesaros [1]

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Friedreich's ataxia (FA) is associated with decreased levels of frataxin mRNA and mature frataxin protein. The best-characterized function of frataxin is in the synthesis of iron-sulfur (Fe-S) cluster prosthetic groups for the enzymes of oxidative phosphorylation, the Krebs cycle and fatty acid breakdown (β-oxidation). Increased incorporation of labeled palmitate into HMG-CoA (the intermediate in either sterol synthesis or ketogenesis) of FA patients suggests increased lipid utilization *via* β-oxidation. The increased β-oxidation produces reduced cofactors, such as $FADH_2$ and NADH that can be utilized to maintain the electrochemical gradient across the inner mitochondrial membrane required for ATP production. Therefore, the increased lipid metabolism observed in FA could play an important role in cellular homeostasis in times of mitochondrial dysfunction. The metabolism of fatty acids in mitochondria is a key physiological process in humans but has not been investigated as a hallmark of FA. Despite this, abnormal lipid accumulation was described in heart and liver cells in mouse models and in the brains of FA patients. In a *Drosophila Melanogaster* model of FA, increased metabolism of fatty acids was reported. Increased activity of inflammatory pathways was also described in different models: sphingolipid synthesis and Pdk1/Mef2 activation in flies and heart tissues from FA patients.

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

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

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

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

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Understanding the role of the RNA-binding protein Pumilio1 in two different neurological diseases

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 207

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Pumilio1 (*PUM1*) is an RNA-binding protein that negatively regulates the levels of its targets. We recently discovered that *PUM1*is important for brain development leading to two different neurological diseases with different phenotypes grouped as Spinocerebellar Ataxia 47 (SCA47) with autosomal dominant inheritances with incomplete penetrance. The early-onset PADDAS *Pumilio1*-Associated Developmental Disability, Ataxia and Seizure causes variable degrees of developmental delay, motor coordination, and sometimes intellectual disability. The adult-onset PRCA *Pumilio1*-Related Cerebellar Ataxia, on the other hand, is a mild, late-onset pure cerebellar ataxia that strikes in the fourth or fifth decade of life (Gennarino et al., *Cell*, 2018). The mutations that lead to these different phenotypes destabilize the protein to different extents: PADDAS-causing mutations reduce PUM1 levels by about 50% in patient cells, whereas PRCA-causing mutations reduce PUM1 by only about 25%. Yet in both cases, only one allele of *PUM1* has a fully functional target-binding domain and the target genes go up at the same way. Why, then, is there such a dramatic difference in phenotype? We hypothesized that PUM1 causes disease via two mechanisms. When PUM1 levels drop 25%, there is loss of target repression. When PUM1 levels drop farther, however, there is too little PUM1 for its interactors to form proper complexes, or the complexes become less stable. To test this hypothesis, we mapped both the PUM1 targetome and its interactome, focusing on the three brain regions where PUM1 is most highly expressed (cerebellum, cortex and hippocampus). Using mass spectrometry, we identified more than 400 different interacting proteins, among which were a large number of RNA-silencing and anaphase promoting complex classes, with some of the interactions restricted to particular brain regions (**Figure 1**). We have validated these interactions in both adult wild-type mice and in patient-derived cell lines. We also found that the mRNA and protein levels of the strongest interactors change according to sex and brain region in *Pum1*$^{-/-}$and*Pum1*$^{+/-}$ mice. These data emphasize the importance of understanding not just the targets of RNA-binding proteins but also their protein partners. Furthermore, our observations support the two-pronged model for PUM1-related diseases.

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

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

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

Methods: A doxycycline-inducible mice model of Fxn deficiency was used (TG mice). Doxycycline (Doxy) was delivered via the chow starting when mice were 9 weeks of age. Cardiac function was evaluated by echocardiography and electrocardiography (ECG). Oxidative phosphorylation (OXPHOS) was measured in isolated heart mitochondria as oxygen consumption (JO_2). Activity of nutrient (mTORC1 and AMPK) and stress signaling (eIF2α) was measured by western blot (WB) as (p)-phospho/total protein ratio. Finally, to evaluate if Fxn-depleted hearts expressed an integrated mitochondrial stress response (ISRmt), qPCR was used to measure transcript levels of ISRmt genes (1C-metabolism, mitochondrial unfolded protein response and amino acid transport/metabolism).

Results: WB detection of Fxn protein in heart mitochondria showed 95% depletion after 8 weeks of Doxy and near complete depletion after 12 weeks. Concerning heart function, surprisingly TG mice had normal/higher ejection fraction, though ECG showed abnormalities. The heart was not hypertrophied and was in fact decreased in mass (relative to tibia length). In heart mitochondria, there was a time-dependent suppression of OXPHOS driven by pyruvate/malate (up to ~50% decrease) or succinate (up to ~40% decrease). However, when fatty acids were supplied, $JO_2$was minimally changed. Interestingly, despite almost maintained fatty acid-driven bioenergetics, levels of ISRmt mRNAs and p-eIF2α were elevated, indicating that Fxn-depleted hearts were not normal. Because a rise in ISRmt mRNAs in other mitochondrial disease models was causally linked to mTORC1 activation, we evaluated this pathway by WB and found evidence for higher activity in TG mice. Finally, few studies have addressed what triggers mTORC1 activity in the context of mitochondrial dysfunction. Because mTORC1 is negatively regulated by AMPK, we evaluated AMPK activity and found p-AMPK levels were decreased in Fxn-deficient hearts. Furthermore, p-eIF2α was also elevated, which would lead to protein translation and explain the lower heart mass.

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

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

Inducing the autophagy pathway as a potential treatment option for spinocerebellar ataxia

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 171

Dr. Maxinne Watchon [1], ***Dr. Luan Luu*** [1], ***Ms. Kristy Yuan*** [1], ***Ms. Katherine Robinson*** [1], ***Prof. Roger Chung*** [1], ***Prof. Garth Nicholson*** [1], ***Dr. Angela Laird*** [1]

1. *Macquarie University*

Machado-Joseph disease (MJD), otherwise known as Spinocerebellar ataxia-3, is a neurodegenerative disease that causes the loss of coordination and control of muscles. This disease is caused by an autosomal dominant inheritance of the ATXN3 gene containing a CAG trinucleotide repeat region. Amongst the healthy population, CAG repeats range between 12-40 repeats, whilst MJD patients harbour greater than 40 repeats. This trinucleotide region encodes for a polyglutamine (polyQ) stretch within the ataxin-3 protein with a pathological hallmark of ataxin-3 positive neuronal intranuclear inclusions. Understanding the disease mechanisms causing the neurodegeneration in MJD patients is limited and due to this, there is no treatment or cure. However, there are several hypothesised mechanisms of disease.

Growing evidence suggests several neurodegenerative diseases share a common feature: accumulation or aggregation of disease causing proteins possibly due to impairment of protein quality control pathway known as macroautophagy. Macroautophagy, hence further known as autophagy, is primarily involved in the degradation of large molecular weight protein, damaged organelles, misfolded protein or toxic aggregated proteins such as ataxin-3. This occurs by the envelopment of these components via a double membranous structure known as an autophagosome. Degradation of the autophagosome's contents is confirmed when it fuses with lysosomes, containing hydrolytic enzymes, known as autophagic flux. Studies have described the co-localisation of autophagy substrates with ataxin-3 positive neuronal intranuclear inclusions in MJD patients. Thus, the main aims of this project were to first explore the baseline levels of autophagy in the transgenic MJD zebrafish. Secondly, to investigate whether autophagy inducer compounds could provide a beneficial effect on these MJD zebrafish.

Protein lysates of zebrafish expressing human ataxin-3 (23Q and 84Q resembling wild-type and mutant ataxin-3) of ages ranging between the larval stages (6-days-old), adult stages (7-months-old) and aging stages (18-months-old) were subjected to western blotting. This was to compare the protein levels of various autophagy substrates. Whilst the larval and adult zebrafish did not reveal any differences between the autophagy substrates, the aging zebrafish showed significant differences between the substrates ($p<0.041$). Secondly, using a candidate-based screening approach identifying autophagy compounds from the literature, we exposed our mutant ataxin-3 zebrafish between 1-6 days of age. One compound, 'Drug Z', was able to rescue a previously described motor phenotype of our mutant ataxin-3 zebrafish. Immunoblotting analysis of 6-day-old MJD zebrafish samples revealed that Drug Z decreased human ataxin-3 levels (full-length and cleaved ataxin-3). These results are supported by decreased levels in autophagy substrates, p62 and lamp2a, from Drug Z exposure.

From these results, autophagy-inducing compounds, like Drug Z, improve the movement of mutant ataxin-3 zebrafish. This may provide a new avenue of treatment not only for MJD, but also for other neurodegenerative diseases.

EXPRESSION SIGNATURES OF THE APOPTOSIS-RELATED BCL2 AND BAX IN BLOOD AND BRAIN OF MACHADO-JOSEPH DISEASE SUBJECTS AND TRANSGENIC MICE

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 182

Ms. Ana Filipa Ferreira [1], ***Ms. Mafalda Raposo*** [2], ***Ms. Filipa Medeiros*** [2], ***Ms. Emily D. Shaw*** [3], ***Prof. Maria de Fátima Brilhante*** [2], ***Dr. Teresa Kay*** [2], ***Dr. João Vasconcelos*** [4], ***Dr. Maria do Carmo Costa*** [3], ***Prof. Manuela Lima*** [2]

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Introduction: Machado-Joseph disease/Spinocerebellar ataxia type 3 (MJD/SCA3) is a currently incurable autosomal dominant inherited ataxia. The MJD's causative gene, *ATXN3*, encodes for the ubiquitously expressed ataxin-3. Expansion of the polyglutamine (polyQ) tract above a pathological threshold leads to a cascade of pathogenic events causing cell death mostly in selected areas of the cerebellum, brainstem, basal ganglia and spinal cord. We have previously reported the decreased ratio of *BCL2*/*BAX* expression in MJD patients, which seems to indicate activation of apoptosis, an important pathway of cell death. Here, we sought to evaluate in more detail if apoptosis induction is indeed a peripheral transcriptional signature of MJD and if it is reflected in post-mortem brains of patients. We, therefore, defined the transcriptional signatures of *BCL2*and*BAX*in peripheral blood in MJD subjects in different stages of disease progression and assessed the expression of these genes at the transcriptional and protein levels in brain areas affected and non-affected by degeneration in MJD patients and controls. To assess whether *BCL2* and *BAX* are differentially expressed in MJD-affected brains over the course of disease, we also carried out parallel investigations on the expression profiles of the homologs of these genes, *Bcl2*and *Bax*, in peripheral blood, pons (affected) and cerebral cortex (non-affected) of hemizygous YACMJD84.2 (Q84) transgenic mice that replicate several behavioral, molecular and pathological aspects of the human disease.

Material and Methods: mRNA and protein levels of *BCL2* and *BAX*and correspondent mouse homologs were evaluated by quantitative real-time PCR and Western blot, respectively. Experiments were conducted using: a) blood samples of preclinical subjects (PC), patients (P1) and sex and age matched controls; b) blood samples of MJD patients (P2, a subgroup of P1) collected at two different moments of disease progression; c) tissue samples from affected and non-affected areas of post-mortem brains of MJD patients and controls; d) blood samples from pre-symptomatic 9 month-old Q84 mice and non-transgenic littermates (wt); e) samples from pons and cerebral cortex of pre-symptomatic 9 month-old and post-symptomatic (early stage) 18 month-old Q84 mice and respective controls.

Results and discussion: *BCL2* mRNA levels were decreased in peripheral blood of MJD carriers (PC and P1) compared to controls, reaching statistical significance for patients. No differences in *BAX* levels or *BCL2*/*BAX* ratio were found between MJD subjects and controls. Noteworthy, *BCL2*/*BAX* ratio was significantly decreased in patients (P1) compared to PC, reflecting the tendency for lower *BCL2* and higher *BAX* levels with disease progression. This trend for decreased *BCL2*/*BAX*in blood was further observed at the protein level in both affected and non-affected brain areas of MJD patients compared to controls, being statistical significant in the non-affected area. The exploratory longitudinal analysis of *BCL2* and *BAX* levels in blood samples of patients (P2) collected at two time points revealed a statistical trend to the increase of *BCL2* transcripts over time. A significant increase of the *Bcl2*/*Bax*ratio at the protein level was further observed in pons of post-symptomatic 18 month-old Q84 mice compared to wt mice.

Conclusions: Overall these results confirm an activation of the intrinsic apoptotic pathway in peripheral blood of MJD patients as well as a similar activation in brain tissues of MJD patients. Moreover, the observed trend for

increased *BCL2*/*BAX* transcript ratio in PC compared to patients, and increased Bcl2/Bax protein ratio in pons of 18 month-old Q84 mice, which display mild motor impairment, may imply that in early stages of MJD there is an attempt to increase cell protection against toxic ataxin-3 by inducing the anti-apoptotic BCL2.

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

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

Mrs. Laura Robles Rodríguez [1], ***Dr. Pablo Calap*** [2], ***Prof. Federico Vicente Pallardó Calatayud*** [1], ***Dr. Pilar Gonzalez-Cabo*** [1]

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Background: Calcium (Ca^{2+}) homeostasis is crucial to maintain a proper physiology in different metabolic processes and signaling pathways. Both mitochondria and the endoplasmic reticulum (ER) contribute to this process through specific protein bridges termed endoplasmic reticulum-mitochondria associated membranes (MAMs). The interaction between these compartments is mainly mediated by the voltage-dependent anionic channel 1 (VDAC1), located in the mitochondrial outer membrane, the inositol-1,4,5-trisphosphate receptor (IP_3R) in the ER membrane, and the chaperone glucose-regulated protein 75, which links the former two. The wide variety of cellular processes MAMs are involved in (lipid metabolism, autophagy, mitochondrial morphology) are usually altered in several neurodegenerative disorders, such as Friedreich's ataxia (FRDA). We have previously described[1] that frataxin-silenced cells showed an impairment in Ca^{2+}buffering, as a consequence of reduced mitochondrial Ca^{2+}uptake capacity. Thus, our aim is to elucidate the role of MAMs in the physiopathology of neurodegeneration and their relevance as therapeutic targets for FRDA.

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

Results: Our findings indicate significative decreased interactions per cell between VDAC- IP_3R and VDAC-GRP75 in cells with frataxin deficiency as compared to the wild type cell line SH-SY5Y. Addition of the vitamin E mimic Trolox improved the number of interactions per cell in FXN-silenced cells, probably due to stabilization of ER-mitochondria interactions through the reduction of lipid peroxidation. Finally, western blot analysis revealed the subcellular location of frataxin in MAMs, offering new insights about its role in these structures.

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

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

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

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

Dr. Sirio Cocozza [1], ***Dr. Teresa Costabile*** [1], ***Dr. Giuseppe Pontillo*** [1], ***Dr. Maria Lieto*** [1], ***Dr. Camilla Russo*** [1], ***Dr. CHIARA PANE*** [1], ***Prof. Alessandro Filla*** [1], ***Prof. Francesco Saccà*** [1], ***Dr. Arturo Brunetti*** [1]

1. *University "Federico II"*

Purpose

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

Materials and Methods

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

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

Results

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

Conclusions

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

PROFILE AND SEVERITY CORRELATION OF ATAXIA PATIENTS IN A TERTIARY CARE INSTITUTE IN EASTERN INDIA

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 111

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- **Introduction and Background** : The clinical, epidemiological profile and progression of ataxia patients in any population is varied and mostly an unexplored area. .
- **Aim and objective of the study** : To find out the epidemiological , clinical spectrum and etiological profile in ataxia predominant group of patients in our population
- **Material and Methods** – This study is conducted in Department of Neurology, Bangur Institute of Neurosciences, in patients with ataxia as predominant symptoms. Patients who had **Vestibulocochlear problems , head injury , structural and vascular causes, arrythmias** , syncope , **Joint, muscle or primary vision problems** were excluded
- **Study method** –By history , clinical examination and relevant investigations , the ataxia is studied according to prevalence, age of onset , mode of onset, duration and progression of the symptoms, Clinical severity(SARA- scale for assessment and rating of ataxia - for cerebellar ataxia) and etiology of the disease causing ataxia is found (with all relevant investigations and genetic analysis when needed with informed consent) and a correlation and exact clinical profile and spectrum of different diseases causing ataxia is determined. Written informed consent was taken from patients and studied protocol was approved by institutional ethics committee for human research , BIN , IPGMER , Kolkata

- **Statistical Analysis -** Categorical variables are expressed as Number of patients and percentage of patients and compared across the groups using Pearson's Chi Square test for Independence of Attributes/ Fisher's Exact Test as appropriate. Continuous variables are expressed as Mean, Median and Standard Deviation and compared across the groups using Mann-Whitney U test/Kruskal Wallis Test as appropriate. The statistical software SPSS version 20 has been used for the analysis. An alpha level of 5% has been taken, i.e. if any p value is less than 0.05 it has been considered as significant
- **Results**: Out of 188 patients studied , 127 patients had cerebellar ataxia(SCA – spinocerebellar ataxia type 2, 3, 1 ,6 and 12 , MSA C- multiple system atrophy cerebellar type followed by Wilsons disease) . 42 patients had sensory ataxia mainly Sensory ataxic variant of Guillain-Barré syndrome(GBS), Miller fischer , CIDP (Chronic immune demyelinating polyneuropathy , CISP - chronic immune sensory polyradiculopathy followed by Sjogrens disease . 19 patients had Mixed cerebellar and sensory ataxia (Multiple sclerosis most common followed by Vitamin E deficiency). The gait abnormality onset (most prevalent) is significantly earlier than the onset of speech ($p < 0.05$). Cerebellar ataxias had higher disability at presentation (higher mean SARA score- 21.87) as compared to mixed ataxias (SARA – 19.68) and gait disability as main indicator. Increase in SARA score was seen more in mixed category (3.64) than cerebellar ataxia alone category (3.01) with significant association($p<0.05$) showing higher disability progression in mixed category of ataxia(highest increase in Stance component – mean increase by 0.55) The estimated mean increase of SARA Total Score(at 6 months follow up) was maximum in MSA C (5.38) followed by Wilsons disease(3.4), Friedreichs ataxia (2.5) and SCA 2 (2.4) . The progression of the severity and disability of ataxia disease is more in MSA C whereas SCA 2 has lesser degree of progression of ataxia (despite being the most severe- highest SARA score-

28.5 at presentation). Whereas Autoimmune diseases(Anti GAD ,Anti Gliadin) ,Multiple sclerosis , Vitamin E deficiency and drugs/toxins related etiologies showed decreasing SARA score trends on 6 monthly follow up(hence improved outcomes in terms of disability) . Increase in SARA score is found to be significantly associated($p<0.05$) with other clinical features(Pyramidal , extrapyramidal, cognitive) followed by duration of illness , and male sex.

- **Conclusion -** Though epidemiological studies are there previously , correlation with SARA score and its associated determinants is still an unexplored area which is attempted in this study to give an insight of the ataxia predominant diseases prevalent in Eastern India for earlier detection and predicting disease progression .

- **Limitations :**Further follow up of the patients with ataxia required to get a clear insight of disease progression.
- **Further Scope :**Early diagnosis and management strategies can be validated for better disease management and prevention of deterioration to make the otherwise debilitating ataxia disorders a preventable one.
- **Conflict of interest**- Nil

Keywords: Ataxia, Cerebellar , SARA Scale

The cerebellar pathophysiology of Autosomal Recessive Cerebellar Ataxia 2 (ARCA2).

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 126

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Coenzyme Q_{10} (CoQ_{10}) is an important lipid present in all membranes. CoQ_{10} is a strong antioxidant, and its most well characterized function is the channeling of electrons to complex III in the mitochondrial respiratory chain. CoQ_{10} biosynthesis occurs in the mitochondria. Mutations in genes coding for CoQ_{10}biosynthesis complex (complex Q) enzymes are causing CoQ_{10} deficiencies, a heterogeneous group of autosomal recessive conditions. ARCA2 is a rare form of early onset mild ataxia due to mutations in the*COQ8A* gene, coding for an ATPase involved in CoQ10 production by regulating complex Q. We previously demonstrated, in a constitutive *COQ8A* KO mice model, Purkinje cell (PC) specific dysfunction in the cerebellum. In order to uncover the molecular mechanism underlying the disease, we aimed at identifying dysregulated pathways in cerebellum. Using a candidate-based approach, we identified elevated glutamate neurotransmission which we hypothesize that leads to excitotoxicity and increased Ca^{2+} levels in PCs. Furthermore, a transcriptomic analysis on laser-dissected PC identified several pathways that are currently being explored. Finally, to further validate the role of PCs in ARCA2, we generated a PC-specific conditional KO mice. These mice are ataxic in agreement with PC specific dysfunction in the constitutive KO model, and also show dysregulated Ca^{2+} metabolism.

Acute loss of frataxin in human IPSC-derived cardiomyocytes is sufficient to cause phenotypic changes associated with cardiomyopathy

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 136

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

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

Cardiomyopathy is the leading cause of premature death in Friedreich ataxia (FA). The availability of patient-derived Induced Pluripotent Stem Cells (IPSCs), which can be differentiated into cardiomyocytes, has made possible the study of the molecular mechanisms of FA pathology, and the testing of experimental therapeutics, in an affected cell type in vitro.

We have optimized a protocol in which normal, IPSC-derived cardiomyocytes at a late stage of differentiation are transfected with an siRNA against frataxin mRNA, using a scrambled siRNA as control, to generate isogenic lines in which the effects of acute loss of frataxin can be studied. One round of siRNA transfection decreased frataxin protein levels to approximately 50% of normal, similar to levels found in carriers. A second round of siRNA transfection decreased frataxin protein levels to 20-30% of normal, leading to cell death in a few days.

Using RNAseq, we analyzed the transcriptomes of four biological replicates in which frataxin was knocked down to below-carrier levels in IPSC-derived cardiomyocytes. Bioinformatic analyses indicated that the acute loss of frataxin was sufficient to trigger profound mitochondrial dysfunction, affecting all complexes of the electron transport chain (ETC), as well as some enzymes of the TCA cycle. Using oxygen-consumption-rate analyses, we confirmed that the maximal and spare respiratory capacities of the ETC in the frataxin-depleted IPSC-derived cardiomyocytes were severely impaired.

IPSC-derived cardiomyocytes in which frataxin was knocked down also exhibited phenotypic changes that are considered hallmarks of cardiac hypertrophy. We measured physiologically relevant increases in cytosolic calcium concentrations, possibly due to the decreased buffering capacity of dysfunctional mitochondria. Consistent with these findings, we found an up-regulation of secreted peptides such as natriuretic peptide B (NPB) and endothelin-1 in the cells with low frataxin.

Finally, we identified sixty-three genes whose expression changes significantly (with a false-discovery rate of zero) in response to acute loss of frataxin. Some of these genes are known to be dysregulated in cardiac hypertrophy but have not previously been linked to FA. We are currently working on experimental confirmation of these bioinformatic data.

A limitation of the present study is that acute loss of frataxin only approximates the more chronic frataxin depletion in patients, thus our data will need to be confirmed in patient-derived, isogenic lines. Nevertheless, the system described herein has advantages, including: 1) facilitation of biological replicates differentiated to a high yield, and 2) elimination of differences due to incomplete or altered differentiation per se.

Mutant PDYN alters Purkinje cell innervation during cerebellar development in spinocerebellar ataxia type 23

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 78

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Spinocerebellar ataxia type 23 (SCA23) is an adult onset neurodegenerative disease caused by mutations in PDYN encoding the opioid precursor protein prodynorphin, which is processed into the opioid peptides α-neoendorphine, dynorphin A, and dynorphin B. Elevated mutant dynorphin A levels in transgenic mice expressing *PDYN*^{R212W} caused a retraction of climbing fibers starting from 3 months of age and subsequent Purkinje cell loss at 12 months of age. To further investigate the effect of PDYN-R212W on Purkinje cell (PC) innervation we examined PC innervation during postnatal development in *PDYN*^{R212W} cerebella.

We found that expression of PDYN-R212W causes morphological alterations consistent with the development of innervation of PCs including reduced climbing fiber translocation along the PC dendritic tree and loss of GABAergic presynaptic terminals on PC somata. This coincided with a reduction of vermal vGlut2 protein levels and an increase in vGlut1 protein levels suggesting an extension of parallel fiber spine territory in *PDYN*^{R212W} mice. Additionally, the vermis of *PDYN*^{R212W} mice showed transcriptional dysregulation of voltage gated calcium channel subunits including the alpha, beta en gamma subunits between 2 and 8 weeks of age. Since these voltage gated calcium channel subunits also play roles in development and plasticity, suggests that PDYN-R212W alters the expression of genes important for the proper development of climbing fibers and PC function.

In conclusion, we demonstrate a developmental role for PDYN in the cerebellum and advocate that dysfunctions in the glutamatergic and GABAergic systems are implicated in the neuropathology of SCA23. Our observations points towards disturbed cerebellar development as a pathological feature of SCA disease and that aberrant PC innervation precedes PC loss causing cerebellar ataxia

Topic: Therapeutic Approaches and Drug Discovery

Targeting of the serotonin (1A) receptor suppresses mutant ataxin-3 pathogenesis in C. elegans

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 290

Mrs. Joana Pereira-Sousa [1], ***Mrs. Ana Jalles*** [2], ***Ms. Daniela Vilasboas-Campos*** [2], ***Ms. Bruna Ferreira-Lomba*** [3], ***Mr. Jorge Diogo Da Silva*** [4], ***Dr. Mark A. Varney*** [5], ***Dr. Adrian Newman-Tancredi*** [5], ***Prof. Patricia Maciel*** [6], ***Dr. Andreia Teixeira-Castro*** [4]

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Two independent hypothesis-free small molecule screenings identified the modulation of serotonergic signaling as a promising therapeutic approach for Machado-Joseph Disease (MJD) or Spinocerebellar ataxia type 3 (SCA3). One study identified an atypical antipsychotic aripiprazole as a modulator of ATXN3 abundancy in MJD cell and animal models. In another study, compound screening in a nematode model of MJD identified the selective serotonin re-uptake inhibitor (SSRI) citalopram, a widely used antidepressant, as a promising candidate drug. Early and chronic citalopram treatment in mutant ATXN3-expressing nematodes and mice restored motility and reduced mutant protein aggregation. Chemical genetics experiments revealed that the effect of citalopram was dependent on the 5-HT transporter SERT but also on post-synaptic 5-HT receptors activity, including the 5-HT1A receptor (5-HT1AR).

The 5-HT1AR activates different signaling pathways and cellular responses depending on which area of the brain is expressed, behaving as a hetero- or autoreceptor. As an autoreceptor, the 5-HT1AR is involved on a negative feedback loop decreasing serotonin production and the firing rate of serotonergic neurons.

In this work, we explored the effect of three 5-HT1AR agonists: NLX-112, tandospirone and aripiprazole, with different affinities and degrees of selectivity for 5-HT1A and other receptors, in a *C. elegans* model of ATXN3 pathogenesis. NLX-112 is a full and highly selective 5-HT1AR agonist which showed positive effects in movement disorders models. Tandospirone is a partial 5-HT1AR agonist that reduced ataxia, pain, insomnia and depression symptoms in spinocerebellar ataxia (SCA) patients. Tandospirone additional targets include the serotonin 5-HT2 and dopamine D2 receptors. Aripiprazole is a dopamine multifunctional/stabilizer agent with partial agonism for serotonin 5-HT1AR and antagonism for 5-HT2AR.

Here we show that treatment with all compounds ameliorated mutant ATXN3-mediated motor dysfunction in *C. elegans*, however different receptors are required for the therapeutic effect observed. In addition to the dependency on 5-HT1AR, aripiprazole requires D2 receptor, whereas tandospirone requires other serotonergic receptors. NLX-112 effect is mostly attributed to the modulation of the 5-HT1AR. This work suggests that activation of serotonergic signaling by the desensitization of the negative feedback loop by chronic stimulation of 5-HT1ARs suppresses MJD pathogenesis but also proposes differential involvement of the post-synaptic receptors in this response.

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

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

Mr. Andrés Vicente [1], ***Prof. Javier Diaz-Nido*** [1], ***Dr. Frida Loria*** [1]

1. *Universidad Autonoma de Madrid*

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

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

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

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

A pharmacological treatment acting on calcium homeostasis improves motor ability and delays Purkinje cell loss in the ARSACS mouse model

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 247

Dr. Andrea Del Bondio [1], Dr. Fabiana Longo [1], Dr. Daniele De Ritis [1], Dr. Paola Podini [1], Dr. Angelo Quattrini [1], Dr. Bernard Brais [2], Dr. Francesca Maltecca [1]

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Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS) is a childhood-onset cerebellar ataxia caused by loss-of-function mutations in *SACS* gene. *SACS* encodes sacsin, a cytosolic protein mainly expressed in neurons with the highest levels in Purkinje cells. Loss of Purkinje cells is indeed the most prominent feature of ARSACS patients and of the *Sacs*$^{-/-}$ mouse model. To date, sacsin function remains largely unknown and no treatments are available for ARSACS.

Remodeling of the intermediate filament-cytoskeleton is one of the earliest consequences of sacsin absence. Both vimentin (in ARSACS patient fibroblasts) and neurofilaments (in neurons) accumulate in the absence of sacsin, forming atypical dense bundles. Alteration of the intermediate filaments is known to affect mitochondrial distribution in different cell types. We thus hypothesized that abnormal neurofilament accumulation in the absence of sacsin may oppose to mitochondrial trafficking on microtubules, thus favouring mitochondrial docking. In agreement, we demonstrated that mitochondrial transport is altered in distal processes of *Sacs*$^{-/-}$ cultured primary Purkinje cells, which show a significant retention of mitochondria in the soma compared to the wild-type. Likely as consequence of defective mitochondrial transport, we found a deregulation of calcium homeostasis in *Sacs*$^{-/-}$ cerebellum, both *in vitro* and *in vivo*. In fact, mitochondria provide ATP to active calcium clearance systems at the plasma membrane and endoplasmic reticulum, but also exert themselves a fine shaping of calcium signals by accumulating calcium into the matrix. Interestingly, a pharmacological treatment with an off-label drug favouring the synaptic glutamate clearance attenuates motor symptoms and delays PC degeneration in *Sacs*$^{-/-}$ mice, at pre-symptomatic stages as well as post-symptomatic stages. This treatment may represent a therapeutic option for diagnosed pre-symptomatic ARSACS patients, but also for patients with overt symptoms.

Our data suggest deregulation of calcium homeostasis as a crucial feature of ARSACS pathogenesis and offer perspectives for disease treatment.

Elucidation of the metabolic signature of Friedreich's ataxia

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

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

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

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

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

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

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

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Friedreich's ataxia (FRDA) is a multisystemic disorder with no known therapies to date, caused by recessive mutations in the mitochondrial protein frataxin (FXN). FXN participates in the biosynthesis of Fe-S clusters and is considered to be essential for viability. Here we report that when grown in 1% ambient O_2, FXN null yeast, human cells, and nematodes are fully viable. Moreover, in a murine model of FRDA, breathing 11% O_2 attenuates the progression of ataxia, whereas breathing 55% O_2 hastens it. This property is unique to FXN amongst the ISCU-NFS1-LYRM4-FXN complex. In human cells, hypoxia restores steady-state levels of Fe-S clusters in both the mitochondria and cytosol. Cellular studies and *in vitro* reconstitution indicate that hypoxia acts through HIF-independent mechanisms that increase bioavailable iron as well as directly activate Fe-S synthesis, thus overcoming the genetic need for FXN. Our work identifies oxygen as a key environmental variable in the pathogenesis associated with FXN depletion, with important mechanistic and therapeutic implications.

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

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

Dr. Laura Rodríguez-Pascau [1], ***Dr. Joaquim Ros*** [2], ***Dr. Pilar Gonzalez-Cabo*** [3], ***Dr. Vijayendran Chandran*** [4], ***Dr. Pierre-Gilles Henry*** [5], ***Dr. Chistophe Lenglet*** [5], ***Dr. David lynch*** [6], ***Dr. Marc Martinell*** [1], ***Dr. Pilar Pizcueta*** [1]

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Friedreich's ataxia (FRDA) is a rare autosomal recessive neurodegenerative disease characterized by progressive spinocerebellar and sensory ataxia, cardiomyopathy, diabetes mellitus and skeletal deformities. It is the most common inherited ataxia and is caused by mutations in the frataxin (*FXN*) gene resulting in reduced levels of frataxin, a small, nuclear-encoded mitochondrial protein. The pathological consequence of frataxin deficiency is a mitochondrial dysfunction including reduced activity of iron-sulphur cluster containing enzymes, mitochondrial iron overload, defective energy production and calcium metabolism, oxidative stress and dys-regulation of mitochondrial homeostasis including mitochondrial biogenesis. Several studies in both cell and animal models have shown that the peroxisome-proliferator activator receptor gamma (PPARγ)/PPARγ coactivator 1 alpha (PGC1α) pathway is dysregulated when there is frataxin deficiency contributing to the disease pathogenesis and supporting PPARγ pathway as a potential therapeutic target for FRDA. Leriglitazone (MIN-102), is a novel selective PPAR gamma agonist with improved profile for CNS diseases. In this study Leriglitazone was tested in vitro in frataxin-deficient dorsal root ganglia neurons, frataxin-deficient cardiomyocytes and fibroblasts from FRDA patients and in vivo in several mouse models of FRDA. The data generated in the different preclinical FRDA related models showed that Leriglitazone is efficacious by increasing DRG neurons survival and decreasing neurite degeneration, compensating the alterations on mitochondrial markers such as PGC1α and improving mitochondrial function, restoring energy production and calcium dysregulation, and ameliorating motor function. Thus, the results support the use of the compound for the treatment of FRDA. Leriglitazone is currently in a phase 2 clinical trial for the treatment of FRDA in Europe and in a phase 2/3 clinical trial for the treatment of Adrenomyeloneuropathy (AMN) in Europe and US.

Treatment with HDAC inhibitor compounds is protective for models of spinocerebellar ataxia

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 165

Dr. Maxinne Watchon [1], Dr. Luan Luu [1], Ms. Katherine Robinson [1], Ms. Isabella Lambert-Smith [1], Ms. Maddy Tym [1], Ms. Kristy Yuan [1], Ms. Hannah Suddull [1], Dr. Emily Don [1], Prof. Roger Chung [1], Prof. Garth Nicholson [1], Dr. Angela Laird [1]

1. *Macquarie University*

Spinocerebellar ataxia-3 (SCA-3, or Machado-Joseph disease, MJD) is a fatal neurodegenerative disease that impairs control and coordination of movement. SCA-3 is caused by presence of a long polyglutamine (polyQ) region within the ataxin-3 protein. Ataxin-3 protein has multiple reported functions, including regulation of transcription and histone acetylation; expansion of the polyQ tract within ataxin-3 may impair these functions.

Here we tested the effect of treating transgenic SCA-3 zebrafish larvae expressing human ataxin-3-84Q with known histone deacetylase (HDAC) inhibitor compounds (sodium valproate and sodium butyrate) from 1-6 days post-fertilisation. Treating the SCA-3 zebrafish with either of the compounds improved the distances swum by the zebrafish and also increased levels of histone acetylation in the SCA-3 zebrafish, suggesting improved transcription regulation. In both cases we also noted an increase in the amount of human ataxin-3 protein expressed by the zebrafish, which would usually be linked with worse outcome for the zebrafish. We therefore hypothesized that treatment with the HDAC inhibitors had also resulted in activation of a neuroprotective pathway that was countering the increased expression of polyQ expanded ataxin-3. To investigate what pathways were activated by treatment with SV we performed proteomics and Ingenuity pathway analysis of protein lysates generated from SCA-3 zebrafish larvae either treated or untreated with SV. This analysis predicted that SV treatment had activated the autophagy protein quality control pathway and the sirtuin longevity signaling pathway. Activation of the sirtuin pathway was confirmed by a finding of increased SIRT1 protein levels in SV treated SCA-3 zebrafish or SCA-3 cell cultures (HEK293 cells expressing human ataxin-3-Q84). SV treatment of the SCA-3 cell cultures also resulted in increased autophagy markers and a nucleus-to-cytoplasm shift of the autophagy substrate selector LC3. In a similar manner, treating the SCA-3 cell cultures with sodium butyrate resulted in a strong induction of the autophagy pathway and increased formation of autolysosomes revealed by immunostaining.

These results suggest protective effects of treatment with two different HDAC inhibitor compounds. Further, they provide the first evidence of sodium valproate inducing activation of the sirtuin pathway and suggest that drugs that target the sirtuin pathway warrant further investigation for the treatment of SCA-3.

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

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

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

Trehalose in Machado Joseph Disease: Safety, tolerability and efficacy

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Background: Machado-Joseph disease (MJD), also known as Spinocerebellar Ataxia type 3, is relatively prevalent among the Yemenite Jewish subpopulation living in Israel. Currently, there is no treatment able to modify the disease progression. Trehalose is a disaccharide with protein stabilizing and autophagy enhancing properties. In animal models of MJD, Trehalose showed reduction of cerebellar lesion size and improved motor function. The purpose of this study was to evaluate the safety, tolerability and efficacy of Trehalose in MJD patients.

Methods: The trial was designed to be a proof of concept, phase 2 study lasting 6 to 12 months, to determine the safety, tolerability and efficacy of weekly IV administration of 15 gr or 30 gr 9% Trehalose solution in MJD patients.. Primary endpoints were safety and tolerability, which were assessed by various clinical and laboratory tests; and secondary endpoints were changes in the Scale for Assessment and Rating of Ataxia (SARA) score, Neurological Examination Score for Spinocerebellar Ataxia (NESSCA), time to do 9 hole peg test and time to do 8 meter walk.

Results: Fourteen clinically and genetically confirmed MJD patients participated in the study, receiving 15 gr (N=8) or 30 gr (N=6) of Trehalose. Trehalose was well tolerated, and no serious drug-related adverse events were noted, The average SARA score, NESSCA and time to do 9 hole peg test (dominant hand) and 8 meters walk for all patients remained stable at six months. Six patients received treatment for as long as 12 months and continued to remain stable on all the above tests.

Conclusions: Based on this trial, IV Ttrehalose seems to be safe in humans. Since there was no placebo group for comparison, the data needs to be interpreted with caution. A further controlled clinical trial is needed to evaluate the efficacy of Trehalose in MJD patients.

Frataxin Function and Quaternary Addition of Small Trojan Tutor Proteins

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

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We propose the quaternary addition of a small tutor protein to modulate the frataxin (FXN) structural dynamics and the activity of the supercomplex involved in Fe-S cluster biosynthesis. We used Ribosome Display technology and the Sac7d protein as the scaffold to select affitins exhibiting the capability of interacting with human FXN. Two binders were obtained, Aff186 and Aff224. Affitins were prepared in *E. coli* and purified. The proteins are structured as judged by CD spectroscopy and temperature-induced unfolding experiments. Affitins showed a marked tendency to dimerize (SEC-FPLC). For both affitins, when DTT was absent, an intermolecular disulfide bond was formed, as detected by ESI-MS. Interaction between affitins and FXN was confirmed and investigated in detail *in vitro* using ELISA, SPR and ITC. Additionally, we were able to map the binding site of Aff186 and Aff224 on the region of the acidic ridge of FXN by analyzing chemical shift perturbations (NMR). The interaction between affitins and FRDA variants (G130V, L198R and W155R) or designed stable FXN mutants was also evaluated. Furthermore, we investigated the affitin-induced modulation of desulfurase enzymatic activity of the human supercomplex NFS1-ACP-ISD11-ISCU-FXN. Moreover, we prepared a Trojan variant of Aff224 to deliver the affitin to the mitochondrial matrix to explore the effect of this molecule in the cellular environment. A new screening with a subsequent round of selection is being carried out to expand the diversity of affitins in order to obtain tutor proteins that can interact with FXN: (a) positively modulating FXN function in the context of the supercomplex; (b) maintaining FXN available to promote activation; or (c) stabilizing mutant variants of FXN for enough time to allow FXN-supercomplex interaction and activation.

Engineered AAVs for efficient delivery to central nervous system

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

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

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

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

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

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

Results. Dose-escalation experiments were carried out with DMF and IMF in C57BL6 mice, by intraperitoneal and oral exposure. Dose ranges were 10mg/kg to 120mg/kg, which because of the mouse's 12-fold faster metabolism overlap the human equivalent doses of 10mg/kg DMF currently EMA-approved for human dosing, i.e. 720mg/day. A dose-dependent increase in frataxin expression and mitochondrial COX4 and Ndufs5 expression was observed in heart and brain and liver tissues from 100% to 400% of baseline in C57Bl6/J mice, and dose-dependent increases in aconitase activity and COXIV activity were also observed. Dose-dependent increase in mitochondrial biogenesis was also observed, though to a lesser extent. The pharmacokinetic (PK) parameters of the novel prodrug IMF were measured, and were superior to MMF, in terms of residence time in the gut and ultimately area under exposure curve. Consistent with this observation of 50-100% improved PK parameters of IMF vs DMF, IMF produced about a 2-fold increased pharmacodynamic potency per weight of drug ingested. Thus IMF was about produced about 2-fold more frataxin expression, COX4 expression, mitochondrial biogenesis, and aconitase activity in heart, muscle and brain than DMF per weight.

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

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

Management of Preexisting Immunity to AAV9 in Friedreich's ataxia

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

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

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

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

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

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

The identification of such peptide provides the proof of principle that it is possible to interfere with the frataxin-RNF126interaction in order to prevent frataxin ubiquitination in living cells. Moreover, it allows to gain insight into the recognition mode between frataxin and RNF126 and identify surfaces and residues involved in the interaction. This information could eventually be developed into the design of small molecule compounds that, by preventing frataxin degradation and inducing its accumulation, could act as effective therapeutics.

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Malisan F, Testi R. 2015. Highly specific ubiquitin-competing molecules effectively promote frataxin accumulation and partially rescue the aconitase defect in Friedreich ataxia cells. Neurobiol Dis 75:91-99.
Rufini A, Fortuni S, Arcuri G, Condo I, Serio D, Incani O, Malisan F, Ventura N, Testi R. 2011. Preventing the ubiquitin-proteasome-dependent degradation of frataxin, the protein defective in Friedreich's ataxia. Hum Mol Genet 20:1253-1261.

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

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

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

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

Alfedi G, Luffarelli R, Condo I, Pedini G, Mannucci L, Massaro DS, Benini M, Toschi N, Alaimo G, Panarello L, Pacini L, Fortuni S, Serio D, Malisan F, Testi R, Rufini A. 2019. Drug repositioning screening identifies etravirine as a potential therapeutic for friedreich's ataxia. Mov Disord 34:323-334.

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

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

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

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

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

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

Materials and Methods: Plasmid pBR325 containing $(GAA)_n$•$(TTC)_n$repeats of varying lengths cloned into the chloramphenicol gene provides a genetic selection for repeat deletion in *E. coli.* The effects of DNA binding or intercalating agents, coralyne, neomycin, and 4-aza-tryptanthrin on cell survival and rates of repeat deletion were measured using standard assays. Agents identified to increase repeat deletion in *E. coli* were then tested in human cells. We are currently performing dose-dependent analysis in three FRDA lymphoblast cell lines from the same family, with two normal alleles (GM15851), a heterozygous line with one expanded allele (GM15849), and a homozygous line with two expanded alleles (GM15850). Cell lines are cultured in RPMI1640 supplemented with Gln, 15% FBS, 1% pen-strep at 37°C in 5% CO_2 for a defined number of generations. Cells are grown in the presence of varied concentrations of different DNA binding agents and samples are taken at different stages of growth for analysis of repeat length by polymerase chain reaction (PCR).

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

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

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

Treatment with sodium butyrate induces autophagy activity in cell culture models of Machado Joseph Disease

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 179

Dr. Luan Luu [1], Ms. Katherine Robinson [1], Dr. Maxinne Watchon [1], Ms. Kristy Yuan [1], Dr. Albert Lee [1], Ms. Hannah Suddell [1], Ms. Alana De Luca [1], Dr. Emily Don [1], Ms. Caitlin Lucas [1], Prof. Roger Chung [1], Prof. Garth Nicholson [1], Dr. Angela Laird [1]

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Machado Joseph Disease (MJD) is a fatal neurodegenerative disease caused by the expansion of the glutamine repeat in the ataxin-3 protein. Normally there are 12-44 glutamine repeats, however in MJD patients there are 60-87 glutamine repeats which causes aggregation of mutant ataxin-3 protein and alters DNA binding. In animal models of MJD, there is evidence of transcriptional dysregulation, including an inhibition of histone acetyl transferase (HAT) activity and hypoacetylation of histone 3 and 4. Sodium butyrate (SB), a salt found naturally within our gut following consumption of legumes and dietary fiber, has been shown to have histone deacetylase (HDAC) inhibitor activity. Furthermore, studies from our lab have shown that treatment with SB rescues the hypoacteylayion of histone 3 in addition to improvements in motor behaviour. Here we report the effect of treating cell culture models of MJD (HEK293 or SH-SY5Y cells stably expressing human ataxin-3-84Q) with SB. We found that treatment with SB rectified levels of histone 3 acetylation in HEK293 cells expressing human ataxin-3-84Q and increased induction of the autophagy quality control pathway, as evidenced by increased levels of the autophagy marker LC3B and decreased levels of the autophagy substrate p62, compared to vehicle treated cells. Further, co-treating the cells with SB and the autophagy inhibitor bafilomycin resulted in increased levels of LC3B compared to following treatment with bafilomycin alone, indicating that SB treatment indeed induces autophagic flux. This is the first time that treatment with SB has been reported to induce autophagy for the treatment of neurodegenerative disease. Hence, we propose treatment with SB may ameliorate disease features of MJD by increasing induction of the autophagy pathway. Functionally, increased induction of the autophagy pathway may aid clearance of protein aggregates and neurotoxic inclusions from cells, in turn reducing neurodegeneration. Following on from this we are currently exploring the effect of SB treatment on the presence of ataxin-3 protein aggregates and cell toxicity in cells expressing ataxin-3-84Q.

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

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

Ms. Elena Britti [1], ***Dr. Fabien Delaspre*** [1], ***Ms. Marta Medina-Carbonero*** [1], ***Dr. Stefka Mincheva-Tasheva*** [1], ***Ms. Arabela Sanz*** [1], ***Dr. Jordi Tamarit*** [1], ***Dr. Joaquim Ros*** [1]

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Introduction: Our group set up two validated cellular models of frataxin deficiency using neonatal rat primary cultures (cardiomyocytes and neurons from dorsal root ganglia-DRG) and lentivirus mediated RNA interference. FXN-deficient cardiomyocytes display disorganized mitochondrial network with evidence of mitochondrial swelling and increased presence of lipid droplets, suggesting impairment of lipid metabolism and/or of respiration [1]. FXN-deficient DRGs present decreased mitochondrial membrane potential (MMP), altered calcium homeostasis and signs of apoptotic cell death [2]. Several evidences suggested that vitamin D could be able to protect FXN-deficient DRGs from neurodegeneration because of anti-apoptotic and neuroprotective effects of Vitamin D (1α,25$(OH)_2D_3$ or calcitriol) [3,4]. Moreover, the last step in the synthesis of calcitriol depends on CYP27B1, a heme-containing mitochondrial enzyme that hydroxylates 25-OHD_3 (or calcidiol) to the active form 1α,25$(OH)_2D_3$ and whose expression is repressed by calcitriol. The activity of this cytochrome is dependent on its coupling with adrenodoxin (ferredoxin), an iron-sulfur protein, to transfer electrons from ferredoxin reductase to CYP27B1 [5] and described to interact with frataxin [6].

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

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

Additionally, we observed a marked increase in CYP27B1 levels observed in frataxin-deficient cultures -thus suggesting low levels of 1α,25$(OH)_2D_3$- were reverted to normal values by calcitriol treatment (**Fig. 2A**). Furthermore, frataxin-deficient neurons display decreased amounts of the iron/sulfur protein FDX1, supporting the hypothesis that calcitriol synthesis should be reduced in these cells. Of note, when these cells were supplemented with calcitriol, the normal levels of FDX1 were restored (**Fig. 2B**).

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

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Fig. 1. Cell survival and neurodegeneration analysis of 5-day-transfected DRGs treated with 20nM 1α,25(OH)2D3 and vehicle, two days after lentivirus transduction. In **A**, the histogram shows the percentage of surviving DRGs treated compared to the vehicle. Survival is analyzed as nº of cells at 5 days compared to the initial value for each condition. In **B**, the histogram shows the decreased percentage of neurite degeneration using the treatments.

Fig. 2. Total lysates of 5-day-transfected DRGs treated with 20nM 1α,25(OH)2D3 and vehicle were analysed by WB. In **A**, it was used CYP27B1 antibody and anti-β-actin as a loading control. In **B**, it was used FDX1 antibody and Coomassie BB stained membrane as a loading control.

Deconstructing the ketogenic diet to treat Friedreich's Ataxia

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

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

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

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

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

Preclinical Translational Research Opportunities at the NINDS

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

Dr. Amelie Gubitz [1]***, Dr. jonathan sabbbagh*** [1]***, Dr. Daniel Miller*** [1]***, Dr. Chris Boshoff*** [2]

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

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

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

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

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

Mr. jixue li [1], ***Dr. Jill Napierala*** [1], ***Dr. Marek Napierala*** [1]

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

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

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

Dr. Gabriela Vilema-Enriquez [1], ***Mr. Robert Quinlan*** [2], ***Dr. Saba Saqlain*** [1], ***Mr. Peter Kilfeather*** [1], ***Dr. Laurent Laboureur*** [3], ***Dr. Irene del Molino del Barrio*** [1], ***Dr. Gabriele Corda*** [4], ***Prof. Ian Blair*** [3], ***Prof. Paul Brennan*** [2], ***Prof. Richard Wade-Martins*** [1]

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Vilema-Enríquez G[*1], Quinlan R[2,3], Saqlain S[1], Kilfeather P[1], Laboureur L[4,5], del Molino del Barrio I[1], Corda G[1], Blair I[4,5], Brennan P[2,3] & Wade-Martins R[1]

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Abstract

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

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

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

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

A retrospective survey of treatment efficacy in glutamic acid decarboxylase antibody (GAD-ab) positive cerebellar ataxia and other neurological syndromes

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 228

Dr. Gilbert Thomas-Black [1], ***Prof. Paola Giunti*** [2], ***Dr. Hector Garcia-Moreno*** [3], ***Dr. Zofia Fleszar*** [1], ***Dr. Rachel Brown*** [4], ***Dr. Michael Lunn*** [5], ***Dr. Mel Hart*** [5], ***Dr. Michael Zandi*** [5]

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Introduction:GAD-ab has long been recognised as a potential cause of cerebellar ataxia, however there has also long been debate about the pathogenicity of GAD-ab and therefore whether exposing these patients to potentially harmful therapies holds any benefit. We undertook a retrospective study in the form of a case note review of GAD-ab positive samples submitted to the National Hospital for Neurology and Neurosurgery in the last 5 years. Our aim being to investigate which therapeutic interventions were of benefit and which syndromes were most amenable to said therapeutics.

Methods

*Study Design*Retrospective case note review and evaluation of service provision.

*Participants*Patients with GAD-ab positive results (>1000 IU) in the last 5 years as measured by indirect ELISA (Euroimmun).

*Analysis*Primary outcome was treatment benefit as demonstrated by an objective measurement e.g. 10m timed walk test or subjective improvement according to the treating clinician. Data were analysed with SPSS v24.0.

Results

In total there were 158 GAD-ab positive patients, of which 100 had titres >1000 IU. This cohort contained 17 cerebellar ataxia (CA) (10.9%), 21 Stiff Person Syndrome (SPS) (13.5%), 26 epilepsy (16.6%), 105 diabetes (66.9%). 72 treatments were administered to 40 out 50 patients with neurological disease in the form of IVIG (62.5% of treated patients), immunosuppression (44.7%), monoclonal antibody (13.5%) or plasma exchange (65.8%). Some benefit was observed in 21 patients (53.8% of treated patients). When the association between positive response to treatment and clinical phenotype was examined, SPS was found to have an OR=12.44 (1.97-78.46, p=0.007) when adjusting for presence of epilepsy and ataxia. Conversely, cerebellar ataxia's OR=0.061 (0.005-0.776, p=0.031), when examined on an individual treatment basis, 5 out of 17 CA patients received steroids which no patient benefited from, 5 received IVIG which was seen to have a clinical response in 1 patient, 8 received a course of plasma exchange of which response was seen in 2, finally one patient received rituximab and responded well to this.

Conclusion

Our data suggest that in GAD-ab positive syndromes the response is associated with the clinical phenotype, with SPS being more amenable to treatment than CA. Given that none of the therapies regularly used in these conditions is without risk, we recommend that in all cases of GAD-ab CA, should immunomodulatory therapy be trialed, quantitative measures such as the SARA and SCAFI be performed at specified time points before and after treatment to enable objective assessment of benefits. This will allow us to better counsel patients as to the possible risks and

benefits of these diverse interventions. Until such standardised, objective data are available we cannot comment on the true efficacy of immunomodulatory therapies in Gad-ab CA.

CRISPR-CAS9 SYSTEMS TARGETING ATXN3 AS THERAPEUTIC APPROACHES FOR MACHADO-JOSEPH DISEASE

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 271

Ms. Sara Lopes [1], ***Mr. Carlos Matos*** [2], ***Mr. André Conceição*** [3], ***Mr. Frederico Pena*** [3], ***Mr. Miguel Lopes*** [3], ***Mr. Rui Nobre*** [4], ***Prof. Clévio Nóbrega*** [2], ***Ms. Ana Vasconcelos-Ferreira*** [5], ***Prof. Neville Sanjana*** [6], ***Prof. Patrick Hsu*** [6], ***Prof. Lukasz Swiech*** [6], ***Prof. Le Cong*** [6], ***Prof. Feng Zhang*** [6], ***Prof. Luis Pereira de Almeida*** [5]

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Machado-Joseph disease (MJD) is an autosomal dominantly-inherited neurodegenerative disorder, caused by an over-repetition of the polyglutamine-codifying region in the *ATXN3* gene. Expanded ataxin-3 is prone to aggregate and disturbs diverse cellular systems, ultimately leading to cell dysfunction and death in specific neuronal populations. To date, no treatment able to reverse or stall MJD progression have been developed. Nonetheless, gene-based therapeutics, including CRISPR-Cas9 systems for gene edition, offer unprecedent perspectives as potential therapeutic approaches for monogenic disorders such as polyglutamine diseases.

To this end, several CRISPR-Cas9 approaches currently allow: i) the permanent inactivation of genes of interest; ii) the correction of pathogenic mutations and iii) the suppression of the transcription of diseased genes. In the first two scenarios, this can be achieved through the use of a Cas9 with catalytic activity and programmable specificities while, on the third case, an inactive Cas9 (dCas9) fused with a transcriptional repressor KRAB, decreases the expression level of the gene.

Here, we designed CRISPR-Cas-based tools for all these applications and tested them on human cell lines and in MJD mouse models. To permanently inactivate *ATXN3* gene we targeted an early exon and tested the editing capability of the system *in vitro*, where it demonstrated its efficiency on gene disruption and consequently reduction of ataxin-3 protein levels, in both HEK293T and iPSC-derived neurosphere cell lines from both controls and MJD individuals. Adeno-associated viral particles encoding these sequences were subsequently delivered in the striatum of a lentiviral-based mouse model of MJD by intracranial injection, where we observed a drastic reduction of the mutant protein, which resulted in decreased accumulation in ataxin-3 aggregates in the striatum, thus preserving neuronal function. This work provides the first *in vivo* evidence of the efficacy of a CRISPR-Cas9-based approach to permanently inactivate the *ATXN3* gene.

To correct the pathogenic mutation of *ATXN3*, we directed CRISPR-Cas9 system to regions both upstream and downstream of the CAG repeat region. In a series of *in vitro* experiments in HEK293T cells, where several guiding sequences were tested, we were able to ablate the intervening genetic sequence, therefore eliminating the disease associated region and originating truncated species of ataxin-3.

In order to transcriptionally repress *ATXN3*, we used the third approach based on dCas9-KRAB which was designed to target either the region of the genome upstream of ATXN3 translation start site, in a non-allele specific manner, and a single nucleotide polymorphism (SNP) in linkage-disequilibrium with the expanded allele (allele-specific strategy). In the non-allele specific approach, we showed that dCas9-KRAB significantly decreased the levels of ataxin-3 mRNA and its protein product in HEK293T cells. On the other hand, when we targeted the SNP in the mutant al-

lele, we were able to reduce the expression of ataxin-3, while leading to no significant decreases in the levels of non-expanded ataxin-3. Importantly, in a MJD transgenic mouse model with severe pathology, the delivery of the silencing agents into the cerebellum ameliorated motor deficits.

Taken together, our work serves as a solid theoretical and practical basis for CRISPR-Cas9-based approach for ATXN3 elimination, correction and transcriptional silencing, as a putative therapeutic avenue in the context of MJD.

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* Sara Lopes and Carlos Matos contributed equally to this work.

THE ANALYSIS OF RECURRENT CASES OF PRENATAL DNA-TESTING OF SPINOCEREBELLAR ATAXIA TYPE I IN YAKUTIA

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 279

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According to the data from the register, out of 1197 patients, 252 of them had spinocerebellar ataxia type I.
Only women from affected families and over 18 years old participated in the study. In the course of 10 years of research of the families affected by SCA1, 80 appointments to prenatal medico-genetic consultations were recorded. During this period, there were 11recurrentappointmentsof pregnant women between the ages of 23 and 32.
Out of 33 appointments to prenatal consultations, prenatal DNA-testing was carried out in 25 cases. According to the gestational ages of the patients, a following distribution was observed: up to 12 weeks of pregnancy inclusively – 19 cases, over 12 weeks of pregnancy - 6 cases, among which there was 1 case of invasive diagnostics in the gestational age of 25.
Following the outcomes of prenatal testing with DNA-negative results, 11 pregnant women were referred to pregnancy prolongation. At the same time in two cases with DNA-positive test results the family made the decision to continue pregnancy. In 12 episodes with DNA-positive test results, the family decided to interrupt pregnancy and only three of them were20 to 25 weeks in their gestational period.
Preliminary awareness of the family in general and young representatives of the families in question in particular, promotes the early appointment to preconceptional and prenatal consultation.
Conducting presymptomatic DNA testing 1-3 years prior to pregnancy in affected women contributes to the active appointment to prenatal counseling.
The recurrent appointments of the families affected by SCA1to prenatal medico-genetic consultations are an indicator of confidence in prenatal diagnosis as a way to achieve the goal of giving birth to healthy offspring and of the effectiveness of prenatal genetic counseling in general.

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

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

Ms. Tennille Rowland [1], ***Dr. John O'Sullivan*** [2], ***Prof. Martin Delatycki*** [3], ***Prof. Jennifer Strong*** [4], ***Dr. Louise Corben*** [3]

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

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

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

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

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

SynTEF1 overrides repressive epigenetic marks at the Frataxin gene

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

Mr. Christopher Brandon [1], ***Dr. Asfa Ali*** [1], ***Ms. Sam Rider*** [1], ***Ms. Sandra Kietlinska*** [1], ***Dr. Mangesh Kaulage*** [1], ***Dr. Aseem Ansari*** [1]

1. *University of Wisconsin, Madison*

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

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

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

Dr. Mangesh Kaulage [1], ***Dr. Asfa Ali*** [1], ***Mr. Christopher Brandon*** [1], ***Dr. Aseem Ansari*** [1]

1. *University of Wisconsin, Madison*

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

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

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

Dr. Hari Padmanabhan [1], ***Dr. Nick Pierson*** [1], ***Dr. John Leonard*** [1], ***Dr. Matthias John*** [1], ***Dr. Marek Napierala*** [2], ***Dr. Yanjie Li*** [2]

1. *CRISPR Therapeutics,* ***2.*** *UAB*

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

AI-powered drug repurposing for rare diseases

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

Dr. Phil Brownjohn [1]

1. *Healx, Cambridge, MA*

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

Fumarates rescue frataxin expression and enhance mitochondrial and aconitase enzyme activity in hearts of FXNKD mice.

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

Dr. Elena Dedkova [1], ***Ms. Claire B. Montgomery*** [1], ***Mr. Chun Kiu Hui*** [1], ***Dr. Alexey A. Tomilov*** [1], ***Prof. Gino Cortopassi*** [1]

1. *UC Davis*

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

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

Results. **Optimal dosing of DMF.**FXNKD and CTL mice were given either vehicle or DMF at 110 mg/kg daily for 3 weeks in peanut butter (vehicle) tabs. This dose of DMF was chosen based on dose-escalation experiments where C57BL6 mice were fed DMF with a daily dose ranging from 40 mg/kg to 160 mg/kg revealing that 110 mg/kg DMF dose led to maximal upregulation in FXN levels and aconitase activity without toxicity. **Optimally dosed DMF rescues FXN expression, mitochondrial and cardiac function.**We demonstrated that FXN expression and activity of mitochondrial aconitase, complex IV in the heart were significantly decreased in FXNKD mice fed dox chow and peanut butter. However FXNKD dox mice treated with DMF showed improved cardiac function which correlated with upregulation in FXN expression levels, fumarate content, aconitase and complex IV activities. Dose-dependent increase in mitochondrial biogenesis was also observed, though to a lesser extent. **Frataxin levels decline in FXNKD blood and recover with DMF.**Furthermore, we showed that FXN levels decline in FXNKD blood in a DOX-dependent way, and FXN levels were rescued in DMF-dosed mice PBMCs. As multiple drugs that used in FA clinical trials do not increase blood FXN, this suggests DMF is unique.

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

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

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

Dr. Chia-Yen Wu [1], ***Dr. Barbara Perez*** [2], ***Dr. Manuela Corti*** [2], ***Dr. Barry Byrne*** [2], ***Dr. Cat Lutz*** [1]

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

Friedreich's ataxia (FA) is the most common form of hereditary ataxias. It is caused by an inherited autosomal recessive expansion of GAA repeats in the promoter region of *frataxin* (*FXN*), resulting in reduced levels of the mitochondrial protein. Hallmark features of the disease include cardiomyopathy and an uncoordinated ataxic gait. There is currently no approved treatment although AAV-mediated gene replacement therapy is a promising strategy. Although many FA mouse models have been developed, most are unsuitable for preclinical studies of gene replacement therapy due to phenotypes too mild to measure. Here, we use a novel conditional knockout mouse model of FA. Mice with floxed exon 2 of the *FXN* gene are crossed with Ckmm-Cre expressing mice, resulting in Cre-mediated deletion of frataxin in heart and skeletal muscle. These mice recapitulate FA cardiac abnormalities on echocardiogram such as decreases in percent ejection fraction and stroke volume as well as hypertrophy. Additionally, mutant mice exhibit peak body weight between 7-8 weeks of age and require euthanasia by 10.5 weeks of age. To evaluate the effect of AAV9-mediated human FXN (hFXN) gene replacement, mice were given a single intravenous dose of AAV9-CBA-hFXN ($6x10^{10}$ vg) on postnatal day 0-2. AAV9-treated animals had similar body weight to wild type littermates, improvements in echocardiogram parameters such as percent fraction shortening, cardiac output, and stroke volume. Importantly, a single dose of AAV9 was able to extend the lifespan of these animals beyond that of untreated littermates. Additional experiments aim to characterize the percent recovery of frataxin protein levels and functional recovery in heart tissue and the effect of dosing animals after symptom onset. These studies support further development of AAV9-mediated frataxin gene replacement as a strategy for the treatment of FA.

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

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

Dr. Rosella Abeti [1], ***Ms. Annalisa Baccaro*** [1], ***Dr. Noemi Esteras*** [2], ***Prof. Paola Giunti*** [3]

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Background. Friedreich's Ataxia (FRDA) is an autosomal recessive neurodegenerative disorder, affecting dorsal root ganglia (DRG), cerebellar dentate nuclei and heart. It is caused by a GAA repeat expansion mutation within the frataxin gene (FXN). This impedes FXN transcription resulting in a progressive decrease of the mitochondrial protein, frataxin. Increased oxidative stress leading to a chronic depletion of endogenous antioxidants affects the survival of the cells and causes neurodegeneration. In particular, cerebellar granule neurons (CGNs) show a significant increase of reactive oxygen species (ROS), lipid peroxidation and lower level of reduced glutathione (GSH). In FRDA, one of the major pathways of oxidant scavengers, the Nrf2 antioxidant pathway, is defective. Previous studies on FRDA-like CGNs showed that the reduced level of frataxin and the oxidative stress induce mitochondrial impairments. By triggering the Nrf2 endogenous pathway pharmacologically we determined whether this could promote mitochondrial fitness and counteract oxidative stress. Methods. In this work, we sought to investigate the beneficial effect of a promising Nrf2-inducer, omaveloxolone (omav), in CGNs from two FRDA mouse models, KIKO and YG8R, and human fibroblasts from patients. By using confocal microscopy we measured oxidative stress and mitochondrial health with and without treatments. Results. We found that CGNs from both KIKO and YG8R presented Complex I deficiency and that omav was able to restore substrate availability and Complex I activity. This was also confirmed in human primary fibroblasts from FRDA patients. Although fibroblasts are not the major tissue affected, we found that they show significant differences recapitulating the disease; this is therefore an important tool to investigate patients' pathophysiology. Interestingly, we found that patient fibroblasts had an increased level of endogenous lipid peroxidation and mitochondrial ROS (mROS), and lower GSH at rest. Omav was able to reverse this phenotype, protecting the cells against oxidative stress. By stimulating the cells with hydrogen peroxide (H2O2) and looking for potential mitochondrial pathophysiology, we found that fibroblasts could not maintain their mitochondrial membrane potential (ΔΨm). Remarkably, omav was protective to mitochondrial depolarization, promoting mitochondrial respiration and preventing cell death. Our results show that omav promotes Complex I activity and protect cells from oxidative stress.

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

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

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

Dr. Rosella Abeti [1], ***Dr. Mittal Jasoliya*** [2], ***Dr. Mark Pook*** [3], ***Mr. Chun Kiu Hui*** [2], ***Prof. Gino Cortopassi*** [2], ***Prof. Paola Giunti*** [4]

1. *Ataxia Centre, Department of Clinical and Movement Neurosciences, Institute of Neurology, University College London, London, United Kingdom.,* ***2.*** *Department of Molecular Biosciences, School of Veterinary Medicine, UC Davis,* ***3.*** *Institute of Environment, Health and Societies, College of Health and Life Sciences, Department of Life Sciences, Division of Biosciences, Brunel University London,* ***4.*** *Ataxia Centre, Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology. National Hospital for Neurology and Neurosurgery, UCLH*

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

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

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

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

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

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

Dr. Yanjie Li [1], ***Dr. Jill Napierala*** [1], ***Dr. Marek Napierala*** [1]

1. *University of Alabama at Birmingham*

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

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

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

Dr. Ankit Shah [1], ***Ms. Zaneta Balantac*** [2], ***Dr. Rohini Sen*** [1], ***Dr. Deborah Hoffman*** [1], ***Ms. Anne Skalicky*** [2], ***Dr. Hao Wang*** [1], ***Dr. Colleen McHorney*** [2]

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

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

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

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

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

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

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

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

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

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

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

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

We set up a *Drosophila* cell-based assay to identify genes that may be able to modify the unusual DNA structures associated to the GAA expansion, and thereby to increase frataxin expression. The experimental strategy was based on the use of two cell lines containing a reporter gene (firefly luciferase), under the effect of ≈300 GAA repeats (the FRDA model cells) or 9 GAA repeats (the control cells). To normalize luminescence measures of firefly luciferase, the two cell lines also express the Renilla luciferase. The experimental design was based on the pACMAN methodology [1] and luciferases expression was controlled by the GAL4-UAS system [2] using a GAL4 driver inducible by copper. Model cells showed 2.5-fold lower luciferase activity (measured as the ratio between firefly luciferase and renilla luciferase luminescence) than the control cells at 1mM CuSO4 that was the working concentration established for copper.

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

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

This work was supported by FARA and ATAXIA Ireland

Pre-clinical study of mesenchymal stem cells transplantation and their secretome administration in an animal model of Spinocerebellar ataxia type 3

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 212

Dr. Andreia Neves-Carvalho [1], Mrs. Barbara Pinheiro [2], Mrs. Joana Correia [2], Mr. Joel Pires [2], Dr. Fábio Teixeira [2], Dr. Nuno Silva [2], Mr. Rui Lima [2], Dr. Susana Monteiro [2], Dr. Sara Duarte-Silva [3], Dr. António Salgado [2], Prof. Patricia Maciel [4]

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Motor uncoordination is one of the symptoms of Spinocerebellar ataxia type 3 (SCA3)/ Machado-Joseph disease (MJD), a progressive and devastating disorder for which there is currently no effective treatment. The low regeneration potential of the Central Nervous System (CNS) represents a challenge for the development of new therapeutic strategies for neurodegenerative diseases, including spinocerebellar ataxias.

In recent years, stem-cell related therapies have been put forward for treating neurodegenerative diseases. Beyond their potential for cell replacement therapies, stem cells also act as immunomodulators and neuroprotectors. Despite some evidence of beneficial outcomes in this context, the short duration of the pre-clinical and clinical trials performed to date, and the fact that most patients regressed to the disease stage prior to treatment, highlights the urgency to assess efficacy of stem cells treatment in a chronic fashion. The purpose of this study was to compare the therapeutic effect of MSC transplantation vs. administration of their secretome in different disease-relevant regions of the CNSin a well-characterized mouse model of MJD (CMVMJD135).

We observed a more marked and sustained benefit upon a single MSC transplantation into the cerebellum when compared with the substantia nigra or spinal cord or with the administration of MSC secretome, that resulted in marginal and transient improvements. Furthermore, our results may also suggest that a single local treatment is not sufficient to produce the desired sustained efficacy, making it therefore important to assess the effect of treatment application in multiple CNS regions, as well as of repeated systemic injections, from an efficacy but also safety standpoint.

In vivo analyses of the ubiquitin-proteasome system in SCA3

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 185

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Spinocerebellar ataxia type 3 (SCA3) or Machado-Joseph disease (MJD) is an inherited neurodegenerative disorder caused by the expansion of a CAG repeat within the *ATXN3* gene resulting in an expanded polyglutamine repeat in the encoded protein ataxin-3. SCA3/MJD therefore belongs to the group of polyglutamine diseases. Up to now, no treatment is available for this disease. One hallmark of this and other neurodegenerative diseases is the formation of inclusion bodies (protein aggregates) in the brain.

Ataxin-3 is a deubiquitinating enzyme and involved in the degradation of proteins via the ubiquitin-proteasome system by editing ubiquitin chains. *Ubiquitin chains can be assembled by the attachment* of *ubiquitins* to different *Lysine residues. Ubiquitins linked via Lysine 48 (K48)*represent the main signal for proteasomal degradation. In order to further study the role of ubiquitination in SCA3, we crossed one of our previously generated mouse models for SCA3 with mice transgenic for mutated ubiquitin (K48R mice). The mutation in K48R mice (Lysine at position 48 is replaced by Arginine) leads to premature termination of K48 poly-ubiquitin chain assembly, hence to the formation of higher amounts of short K48-linked ubiquitin chains.

We used RotaRod and CatWalk tests to measure the motor-coordinative abilities and observed that transgenic SCA3 mice which simultaneously express the mutant ubiquitin transgene (MJD/K48R) showed an alleviated motor phenotype compared to single transgenic SCA3 mice.

To investigate the cause of these improved motor abilities in detail we performed immunoprecipitation and Western blot analyses. Aggregate load was checked via filter trap and immunohistochemistry. We measured the proteasomal activity and distribution of proteasomal subunits using immunohistochemistry. We hypothesize that the presence of higher amounts of short K48-linked ubiquitin chains leads to a higher proteasomal turnover of the expanded transgenic ataxin-3 protein resulting in the alleviated phenotype in MJD/K48R mice. We aim to further explore the feasibility to translate this approach into a therapeutic strategy.

The study was supported by the National Ataxia Foundation (Young Investigator Award) and by the Medical Faculty Tuebingen (Forschungsorientierte Gleichstellungsförderung).

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

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

Mr. Chun Kiu Hui [1], ***Dr. Sandipan Datta*** [1], ***Dr. Mittal Jasoliya*** [1], ***Dr. Elena Dedkova*** [1], ***Prof. Gino Cortopassi*** [1]

1. *UC Davis*

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

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

Results. Dose-escalation experiments were carried out with DMF in C57BL6 mice, by intraperitoneal and oral exposure (gavage and peanut butter tabs). Dose ranges were 10mg/kg to 320 mg/kg, which because of the mouse's 12-fold faster metabolism overlap the human equivalent doses of 10mg/kg DMF currently EMA-approved for human dosing, i.e. 720mg/day. A dose-dependent increase in frataxin expression and mitochondrial COX4 and Ndufs5 expression was observed in heart and brain and liver tissues from 100% to 400% of baseline in C57Bl6/J mice, and dose-dependent increases in aconitase activity and COXIV activity were also observed. Dose-dependent increase in mitochondrial biogenesis was also observed, though to a lesser extent.

Conclusion. These results demonstrate that oral DMF dose-dependently induces frataxin expression, mitochondrial gene expression, aconitase activity and mitochondrial biogenesis in C57Bl6 mice. Furthermore, the 120mg/kg maximally effective dose of DMF in mice overlaps the human equivalent dose of 10mg/kg *that is currently approved for human dosing*,i.e. 720mg/day. Thus, the same pharmaceutical ingredient, DMF, that has been dosed at 480mg/day (as Tecfidera) for Multiple Sclerosis, and at up to 720mg/day (as Skilarence) for psoriasis in more than 200,000 humans worldwide, is effective in raising frataxin and other mitochondrial proteins at the same bioequivalent dose in mice.

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

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

Dr. Catherine Gérard [1], ***Dr. Nathalie Majeau*** [1], ***Mr. Pouire Yameogo*** [1], ***Prof. Jacques P. Tremblay*** [1]

1. *Université Laval*

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

Modeling cardiomyopathy in Friedreich ataxia with patient-derived cardiomyocytes

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

Dr. Chicheng Sun [1], ***Ms. Deena Qadir*** [1], ***Ms. Vivian Villegas*** [1], ***Dr. Owen Wallace*** [1], ***Dr. John Graef*** [1]

1. *Fulcrum Therapeutics*

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

Topic: Genetics of Disease

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

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

Dr. Sathiji Nageshwaran [1], ***Prof. George Church*** [1]

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

Novel genetic modifiers of somatic CAG repeat instability

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

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Identification of genetic modifiers of somatic CAG repeat instability by in vivo CRISPR-Cas9 genome editing

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

Here, with the goal of dissecting the mechanism(s) underlying somatic CAG repeat expansion we report the development of a CRISPR/Cas9-based platform to identify novel instability modifiers *in vivo*. To this end, we generated HD mice (*Htt*Q111) that endogenously express Cas9, and targeted known modifier genes, that either enhance or suppress somatic CAG expansion based on genetic knockout studies. Following a single tail vein injection of AAV8 or PHP.B-based viruses carrying sgRNAs against the gene of interest, we confirmed strong liver and brain transduction, detected a high frequency of inactivating mutations at target sites, and successfully suppressed or hastened somatic CAG expansions in HD mice. We also report on using this CRISPR/Cas9-based platform to: 1) investigate new candidates genes identified as part of a large HD age at onset Genome Wide Association Study (GWAS), as potential modifiers of CAG instability; 2) probe more broadly the role of other candidate DNA repair genes in this disease-relevant process; and 3) obtain a better understanding of gene-gene interactions and dependencies.

Together, this strategy has already resulted in the successful identification of novel modifiers of the somatic CAG expansion process, as well as providing significant insight into mechanisms of somatic CAG instability and novel targets for therapeutic intervention.

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

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

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

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

CCG•CGG interruptions found on highly penetrant SCA8 alleles increase RAN protein levels and protein toxicity

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 245

Dr. Hannah Shorrock [1], ***Dr. Barbara Perez*** [2], ***Dr. Monica Banez Coronel*** [1], ***Ms. Tammy Reid*** [1], ***Dr. Yoshio Ikeda*** [3], ***Prof. Christopher Gomez*** [4], ***Dr. Thomas Bird*** [5], ***Dr. Tetsuo Ashizawa*** [6], ***Dr. John Day*** [7], ***Dr. Lawrence Schut*** [8], ***Dr. Alfredo Brusco*** [9], ***Dr. Lis Hasholt*** [10], ***Dr. Jorgen Nielsen*** [11], ***Prof. S H Subramony*** [2], ***Prof. Laura P. Ranum*** [1]

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Spinocerebellar ataxia type 8 (SCA8) is a neurodegenerative disorder caused by a bidirectionally transcribed CTG•CAG repeat expansion. SCA8 has a widely variable age of disease onset and reduced penetrance, whereby many individuals that carry the expansion mutation do not develop the disease. Although SCA8 is transmitted in a dominant manner with many asymptomatic expansion carriers, the reduced penetrance of the mutation makes SCA8 most often appear sporadic, with no apparent family history of disease. Here, we present the largest study to date of SCA8 patients from 71 independent families. Utilizing this cohort to investigate the reduced penetrance, we found that families with two or more affected individuals carry CCG•CGG interruptions within the SCA8 repeat tract at a higher frequency than families who present with only a single affected individual. We show that these interruptions result in increased steady state levels of SCA8 RAN polyAla and RAN polySer proteins. The presence of CCG•CGG interruptions also increases the toxicity of the ATG-initiated polyGln protein by the introduction of arginine interruptions, and of the RAN polySer protein by the introduction of glycine interruptions. These data show sequence interruptions increase RAN proteins levels and the toxicity of *ATXN8*-derived proteins, providing a molecular link between CCG•CGG interruptions and the increased penetrance found in families with these alleles.

Somatic mosaicism in human SCA1 post-mortem brains

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 237

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Background: Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant neurodegenerative disorder caused by the expansion of a polyglutamine (polyQ) encoding CAG tract within the *ATXN1*gene. Instability of the CAG repeat is a prominent feature of the polyQ diseases. Early studies on somatic mosaicism in Huntington's disease (HD) disease suggested that the variation in the size of the CAG expansions in different tissues of the same individual may play a role in the tissue-specific effects of the *HTT*gene (Telenius *et al.*, 1994). Subsequent studies in SCA1 contradicted this, demonstrating that the neuronal patterns of somatic mosaicism in SCA1 patients mirrored those of HD (Chong *et al*, 1995; Zühlke*et al*., 1997). This implies that tissue-specific factors, such as the DNA repair genes, are modulating the stability of the expansion in a tissue-specific manner. In order to test this hypothesis, we examined the regional somatic mosaicism and DNA repair gene expression in four SCA1 brains.

Materials and Methods: Four SCA1 post-mortem brains and matching blood were available for analysis. DNA was extracted from eight brain regions (frontal cortex, temporal cortex, occipital cortex, pontine nucleus, caudate nucleus, pons, medulla and cerebellum) and blood. The DNA for two SCA1 brains was fragment sized, cloned and sequenced, as previously described (Menon *et al*., 2013). DNA for all four SCA1 brains was also analysed using novel Illumina MiSeq sequencing techniques. RNA was extracted from the same eight brain regions for the four SCA1 patients and four controls. RT-qPCR was used to analyse the expression levels of a panel of DNA repair genes previously reported to impact CAG repeat instability.

Results: Clone sequencing demonstrated the stability of the CAG repeat in the cerebellum, with no sequence interruptions observed in the SCA1 patient brains and corresponding blood that could influence repeat stability. This was confirmed by Illumina MiSeq sequencing with the CAG repeat revealed as presenting with considerably fewer somatic expansions in the cerebellum than all other brain regions, contrary to their association with SCA1 pathology. To address this discrepancy, we examined the expression levels of DNA repair genes within these regions. We observed a significant increase in*PMS2*expression in the frontal cortex, temporal cortex, occipital cortex, medulla and cerebellum in pooled SCA1 patients compared to controls. *MSH6*was also significantly increased in the cerebellum relative to cortical brain regions, both in patients and controls.

Conclusions: Repeat configuration is consistent throughout different tissues in SCA1 patients. Somatic mosaicism does not correlate with specific tissue neurodegeneration in SCA1 brains. Here we present novel somatic mosaicism and DNA repair gene expression analyses in human post-mortem brains for expanding our understanding of specific regional vulnerability in polyQ disorders.

Investigation of variants in genes of the ataxin-3 cleavage pathway as age at onset modifiers factors in MJD/SCA3 cohort

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 200

Ms. Ana Carolina Martins [1], ***Mrs. Mariana Rieck*** [2], ***Prof. Maria Luiza Saraiva-Pereira*** [2], ***Prof. Laura Jardim*** [2]

1. *UFRGS,* ***2.*** *HCPA & UFRGS*

Background: Machado–Joseph disease, also known as spinocerebellar ataxia type 3 (SCA3/MJD), is caused by an expanded CAG (CAGexp) repeat at *ATXN3*, which translates into a polyglutamine tract (polyQ) within the ataxin-3 protein. SCA3 is the most prevalent form of ataxia in the world, and Rio Grande do Sul (Brazil) is the local with the highest relative frequency, representing 78% of all local spinocerebellar ataxias. The CAGexp explains around 55% of the variation in the age at onset (AO). The remaining variability in AO might be explained by factors such as family (~10%), population-specific factors (8.3%), presence of normal/large *ATXN2* alleles (27–33 CAG repeats) (PMID 30337442). Therefore, we have concentrated efforts in order to find new modifiers of AO. Proteolytic cleavage has been proposed as a key event in the molecular pathogenesis of SCA3/MJD. Ataxin-3 is substrate for two main families of proteases: caspases and calpains. Calpain-mediated proteolysis promotes ataxin-3 translocation to the nucleus, aggregation, cell injury and neurodegeneration; degradation of mutant ataxin-3 by calpain seems to be more efficient than that of wild-type ataxin-3, and increased proteolytic activity results in a more severe and accelerated progressive neurological phenotype. In this work, we aimed to evaluate the relation between AO of SCA3/MJD carriers and single-nucleotide polymorphisms (SNP) in *CAPN2* (rs17599) the gene that translates into Calpain-2, and *CAST* (rs27852 and rs1559085 variants).

Methods: The AO of the first symptom were predicted for each patient according to the CAGexp in the overall SCA3/MJD cohort from South Brazil (520 subjects), so that deltas between the predicted and observed AO (deltaAO) were obtained and treated either as a continuous variable, as well as used to stratify the group into early (observed AO before the 25 percentile of the predicted AO), average and late (after the 75 percentile) AO subgroups. Distribution of SNP genotypes was compared between AO subgroups by chi-square tests, while associations between AO and *CAPN2* and *CAST* genotypes were tested by ANOVA.

Results: Two hundred forty SCA3/MJD symptomatic subjects whose data on AO was available were included: 125, 32 and 104 subjects were classified as early, average and late onset, respectively. Genotypes of *CAPN2* rs17599, *CAST* rs27852, and *CAST* rs1559085 did not present different proportions of subgroups AO (ns, chi-square), nor differences in their mean AO (ns, ANOVA). AO distributions were not different when the alleles were analyzed in a dominant model (t-test). However, subjects carrying simultaneously the CC genotype rs27852 and the allele G at rs1559085 in the *CAST* gene had a mean AO of 38 (±14,29) years, while AO of the remaining subjects was 34 (±13,02) years (p=0.057).

Discussion: These results suggest that a haplotypic conformation of *CAST* might be related to modulation of SCA3/MJD phenotype - specifically, C at rs27852 and G at rs1559085 were associated with an almost significant delay in AO. This haplotype might be linked to a functional change in calpastatin, at least in our cohort, ie, to an increased effect of calpastatin over calpain inhibition. Further studies, such as those obtained in other cohorts, are important to clarify this association.

Further insights in the combined effect of modifiers of age at onset in Spinocerebellar ataxia type 3/Machado-Joseph disease

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 197

Ms. Rafaella Mergener [1], ***Dr. Gabriel Vasata Furtado*** [1], ***Dr. Eduardo De Mattos*** [1], ***Prof. Vanessa Bielefeldt Leotti*** [1], ***Prof. Laura Jardim*** [1], ***Prof. Maria Luiza Saraiva-Pereira*** [1]

1. *HCPA & UFRGS*

Spinocerebellar ataxia type 3, or Machado-Joseph disease (SCA3/MJD), caused by an expansion of CAG tract (CAGexp) in *ATXN3* gene. This expansion results in protein with a polyglutamine (polyQ) tract which is prone to aggregation. An inverse correlation between CAGexp length and age at onset (AO) of the disease can be noted. However, it can explain on average just 55.2% of the AO variation, which suggests that the disease is modulated by other factors. The search for phenotype modifiers is extensive, and so far, a recent work demonstrated that combining length of CAGexp and the length of CAG repeats at *ATXN2* can explain up to 63.0% of AO variation. Somatic instability has been postulated as cause of disease anticipation over generations, and it is attributed to the actions of DNA repair proteins as well as the individually associated variants once that it occurs in post mitotic neurons. This fact suggests that variants in DNA repair genes might modify AO in polyQ diseases. A genome-wide association study in patients with Huntington's Disease (HD) found significant association between AO to different genes of DNA repair pathways. One relevant association signal was found at two strong candidate genes: *FAN1* and *MTMR10*. Into this signal we can find the SNP rs3512.SCA3/MJD is the most common form of dominant ataxia worldwide, reaching 78.4% of all diagnosed ataxias in the South region of Brazil. In order to contribute to the understanding of factors that modulate AO in this disease, in this study we investigate the role of variant rs3512 as a modifier of AO taking in consideration the effect of *ATXN2* genotypes in a Brazilian cohort of SCA3/MJD patients. A total of 144 SCA3/MJD patients were included. The outcome was AO, defined as age at the first symptom. A control group composed by 50 unrelated healthy individuals was also evaluated. The CAG repeat length was performed by the polymerase chain reaction (PCR) using fluorescent labeled primers flanking the CAG repeat at the *ATXN3* and *ATXN2* genes, followed by capillary electrophoresis. The rs3512 genotyping was performed using TaqMan SNP Genotyping Assay (C_483247_10). We have shown a tendency of association of rs3512 with AO in SCA3/MJD patients, in the same direction that was described previously. In this study, on average, subjects carrying same CAGexp length at the *ATXN3* gene and at least one C allele at rs3512 began the disease 2.44 years later (p=0.052; ANOVA), Showing an even higher effect of the study by Bettencourt and coworkers (2016) that demonstrate a delay of 2.156 years in AO of first symptom for each allele C of individuals with SCA3/MJD. Along with *ATXN2* and CAG length, the protector effect of minor allele gets more prominent showing that, patients with G/G genotype started the disease 2.58 years earlier (p=0.036, mixed model analysis). In the same way, AO of individuals with a short *ATXN2* allele was 4.25 years later (p=0.006; mixed model analysis) when adjusted by the allele. There is no association between genotypes at rs3512 and *ATXN2* groups (p=0.902; chi-square), which indicates that those factors have independent effects. In the present cohort, the *ATXN3* CAGexp explains 65.9% (p<0.001; linear regression) of AO variation. Adding the effect of rs3512, it goes up to 66.5% (p<0.001; linear regression). On top of that, *ATXN2*effect raises this figure to 68.0% (p<0.001; linear regression). Considering allele frequencies distribution, there were no differences found between patients and controls. A frequency of 22% of minor allele was estimated in our local controls cohort, a previously unreported data in the Brazilian population. Therefore, frequency determined in our controls is between frequencies of Latin America (17-18%) and Europe (30-33%). This data might be related to an estimated high rate of European ancestry in the South region of Brazil. Among SCA3/MJD individuals, frequency of minor allele is higher (24.3%) and closer

to the European frequency. This outcome can be due to a founder effect of Portuguese (from the Azorean islands) in our SCA3/MJD cohort.In summary, we demonstrate AO modulation when placing *FAN1* as part of a more complex scenario that can contribute to the onset of SCA3/MJD. Combining effects are very likely to be involved in disease modulation. Data presented here emphasizes the relevance of replicating studies in populations from different ethnic background once that a universal correlation might not be applicable to all subjects at risk considering that clear geographical and/or ethnic differences can be seen on the effect of CAGexp on AO.

Biallelic mutations in histidyl-tRNA synthetase (HARS) cause a novel ataxia-related phenotype in two families

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 149

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Background and purpose

Aminoacyl-tRNA synthetases are ubiquitously expressed and highly conserved enzymes that catalyze the conjugation of tRNA to cognate amino acids in the cytoplasm (ARSs), in mitochondria (ARSs2) or both. To date, autosomal recessive mutations in histidyl-tRNA synthetase (*HARS*) have been associated with Usher syndrome type III, which is characterized by motor defects, progressive retinal impairment, and hearing loss in childhood. Autosomal dominant variants have been linked to the peripheral neuropathy Charcot-Marie-Tooth disease type 2W. Here we describe three patients in two unrelated families showing biallelic mutations in *HARS* without any previously reported *HARS*-related phenotype. We present genetic, phenotypic and multi-functional analyses indicating mutations in *HARS* as cause of the disease in our patients.

Methods

Targeted multigene resequencing panel (Nimblegen, Roche), encompassing 194 genes related to inherited ataxias, and whole-exome sequencing (TruSightOne, Illumina) were performed in patient 1/patient 2 and patient 3, respectively using a NextSeq500 Illumina platform. Ingenuity Variant Analysis (Qiagen) suite was employed for variant annotation following an in-house validated pipeline. Prior to NGS application, all the patients had been tested for pathological expansions in SCA1, 2, 3, 6, 7, 8, 12, 17 and for the intronic GAA expansion in *FXN*. All mutations were confirmed by Sanger sequencing. Multiple *in silico* tools (including SIFT, PolyPhen-2, MutationTaster, FATHMM-MKL, MetaSVM, MetalR, GERP, LRT, Mutation Assessor, Provean and CADD) were used to predict the impact of mutations on the protein function. cDNAs and whole cell lysates were obtained from primary fibroblasts harvested from diagnostic skin biopsies in the three patients, and were used to assess mRNA levels in real-time qPCR and steady state protein levels in Western blotting using an anti-HARS monoclonal antibody (Abcam). Standard yeast complementation assays were performed to determine if each allele has a loss-of-function effect *in vivo*, and HARS aminoacylation activity was measured in fibroblasts extracts.

Results

Patients 1 and 2 are sisters and present clinical features of congenital ataxia with mild mental retardation and dystonic postures. Patient 3 is a boy presenting spastic ataxia gait, upper limb dysmetria, progressive dysarthria, atetoid movements, and a slight mental retardation. Brain MRI showed a severe cerebellar atrophy in patient 3 and a moderate enlargement of cerebellar folia in patient 1. Hearing and retinal functions were normal in the patients and there was no evidence of peripheral nerve involvement. Massive parallel approaches in next-generation sequencing (NGS) revealed that both patient 1 and 2 harbour a missense (c.1393A>C, p.I465L, on the paternal allele) and an in-frame insertion (c.910_912dupTTG, p.L305dup, from the mother), whereas patient 3 harbours a paternal frameshift (c.730delG, p. V244fs*6) and a maternal missense (c.616G>T, p.D206Y). Whilst the p.I465L has a very

low frequency (<1/10^5) the other variants are new and absent in the gnomAD polymorphic database. Besides, both p.D206Y and p.I465L are predicted to have a deleterious effect on the protein function by most of *in silico* algorithms we tested. Quantitative PCR and Western blotting indicated a significant reduction of HARS mRNA and protein expression, respectively in all patients. Yeast complementation analysis of *HARS* variants revealed that p.V244fs*6 and p.L305dup are consistent with a functional null allele, whereas colonies harbouring p.D206Y and p.I465L mutations showed a growth rate comparable to the controls. HARS aminoacylation assay measured in patients' cultured skin fibroblast revealed a significant reduction of enzyme activity in all patients consistent with all four alleles having a loss-of-function effect.

Discussion

We found novel biallelic mutations in *HARS* in three patients with a not yet described *HARS*-related phenotype through the application of NGS technologies. All mutations are rare and predicted to have a significant effect on protein function. mRNA and protein expression levels were dramatically reduced in skin fibroblasts from the patients, suggesting a functional impairment of both alleles. Studies in yeast showed that two of the four mutations enact a loss-of-function effect and enzyme activity studies on patient cells revealed that all four mutations likely impact tRNA charging. Thus, the inheritance pattern, the nature of the identified mutations, and our functional studies all support the pathogenicity of each allele in the identified patient phenotype.

Conclusion

We found novel biallelic mutations in *HARS* in three patients from two different families lacking the usual *HARS*-related phenotypes. Combined genetic, phenotypic, MRI, *in silico*, *in vitro* and *in vivo* analyses corroborate the hypothesis that the mutations we report are the cause of a novel ataxia-related recessive phenotype in our patients.

STUB1 rare variants are in excess in SCA patients: causal variants, risk factors, or both ?

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 180

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STUB1 mutations have first been described among autosomal recessive ataxias (SCAR16). More recently, a dominant phenotype has been reported with early cerebellar cognitive-affective syndrome and SCA48, a late onset spinocerebellar ataxia (SCA). Here we present 30 SCA families presenting with heterozygous *STUB1* variants, frequently associated with a severe cognitive impairment.

We initially observed a dominant SCA family in which the index case presented with an almost isolated and severe frontal syndrome. His neuropsychological examination revealed a severe and complete cerebellar cognitive affective syndrome (CCAS) with dorsolateral (dysexecutive syndrome) and orbito-frontal (behavior trouble) deficits. This phenotype was associated with ataxic gait, dysmetria of upper limbs and a cerebellar dysarthria, SARA scored at 26/40 at age 69 after 15 years of disease progression. His daughter noticed dysarthria at age 38. Her neuropsychological examination revealed mild but definite dysexecutive, orbito-frontal and spatial cognition deficits. A second family displayed a "Huntington disease-like" presentation: predominant behavior changes with emotional lability and aggressivity, associated with mild gait ataxia and dysarthria. Exome analysis revealed the same dominant *STUB1* variant (c.141A>G p.Y49C) in both families. Variant segregation with the disease was confirmed.

Based on these reports, a screening of *STUB1* genetic variants was performed in 499 patients from 447 SCA families who previously underwent a whole exome sequencing (WES). Rare variants predicted to be pathogenic were filtered (minor allele frequency < 0.001 in gnomAD 2.1.1; CADD score > 20). Using these criteria, candidates variants were observed in 30/447 families (~7%), most of these variations being absent from public databases. A majority of these variations were missense mutations, but also included substitutions with stop-gained, and frameshift variations. The mean CADD score was 27.35. Of note, 5 variants of *STUB1* were recurrent in unrelated families, 4 of them being absent from the gnomAD database. All variants were validated by Sanger sequencing. Whenever possible, segregation with the disease was confirmed, arguing in favor of considering *STUB1* as the causal gene in these families in absence of other candidate variants. In addition, a causal gene was previously identified in 4 families (*AGFL2, CP, ATM, PRKCG*) suggesting that *STUB1* variants could act as a risk factor in addition or only in combination with the other variant.

Because of the cognitive phenotype, we performed a screening of WES data obtained from 101 patients suffering from frontotemporal dementia (FTD) to compare frequencies of *STUB1* variants with the ataxia cohort. The same criteria of selection were applied. For 13 patients a FTD gene was previously identified (*C9ORF72, GRN, MAPT, TARDPB*, and *VCP*). Strikingly, and conversely to what has been observed in patients with ataxia, we detected only one potential candidate variant in FTD. The segregation analysis was not feasible in this single family.

Altogether, these findings are in favor of a strong enrichment in *STUB1* rare variations in SCAs compared to FTD (30/447 versus 1/101, p= 0.018), Functional studies are needed to better characterize the role of these variants. From the original description of SCAR16 and SCA48, this work strongly suggests that *STUB1* genetic variations may represent a cause of dementia associated with SCA.

Several phenotypes one gene: AFG3L2, when genetics garble the rules.

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 249

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Spastic ataxia-5 (SPAX5) is an autosomal recessive disorder, characterized by early-onset neurodegeneration, myoclonic epilepsy, dystonia, ptosis and oculomotor apraxia.

The causative gene is AFG3L2, encoding a protein subunit that forms the m-AAA protease (matrix-ATPase associated with diverse cellular activities), a multimeric complex bound to the inner mitochondrial membrane, crucial component of the mitochondrial protein quality control system. Heterozygous mutations of AFG3L2 cause the autosomal dominant form spinocerebellar ataxia type 28 (SCA28).

So far, only four SPAX5 patients have been described, reporting a huge clinical variability.

Here, we report the first compound heterozygous SPAX5 patient reporting two *in-trans* variants in the AFG3L2 gene: c.1847A>G (p.Y616C) and c.1976C>T (p.A659V) (maternally and paternally inherited, respectively). The patient was brought to medical attention at eight months of age when he was unable to sit unsupported; he had oculomotor apraxia and developed myoclonic seizures at age 18 months. He showed *epilepsia partialis continuans* (EPC) starting at age 2.5 years.

As already shown in the literature, SPAX5 patient parents did not show any sign of neurodegeneration nor oculomotor signs, despite carriers of an AFG3L2 mutant allele.

We documented the mitochondrial phenotype of patient-derived primary fibroblasts, showing a slightly reduced mitochondrial membrane potential in compound-heterozygotes patient compared to healthy controls and an increased SOD2 level, suggesting an increased oxidative stress in SPAX5 cells. Muscle biopsy-derived mitochondrial enzyme analysis revealed mild Complex I deficiency. No alteration in mitochondrial network was remarkable, showing patient cells having mitochondrial axes length comparable with controls fibroblasts.

In conclusion, we describe the first compound heterozygous SPAX5 patient, carrying one variant in exon 16 of AFG3L2, the SCA28 mutational hotspot. Our data further corroborate the idea that mutations in AFG3L2 may have a hypomorphic and variable effect, linked to the affected residual and related number of functional (or partially functional) m-AAA hexamers able to be formed.

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

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

Dr. Alexander Brown [1], Dr. Michael Parkinson [1], Dr. Ese Mudanohwo [1], Dr. Robyn Labrum [1], Dr. Mary Sweeney [1], Prof. Paola Giunti [1]

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

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

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

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

Bioinformatics-Based Identification of Expanded Repeats: A Non-reference Intronic Pentamer Expansion in RFC1 Causes CANVAS

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 156

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Maŕıa Garć ıa Barcina,10 David P. Breen,11,12,13 Andrew M. Chancellor,14 Phillip D. Cremer,15,16 Martin B. Delatycki,7,17Brent L. Fogel,18 Anna Hackett,19,20 G. Michael Halmagyi,21,22 Solange Kapetanovic,23 Anthony Lang,24,25 Stuart Mossman,26 Weiyi Mu,6 Peter Patrikios,27Susan L. Perlman,28 Ian Rosemergy,29 Elsdon Storey,30 Shaun R.D. Watson,31 Michael A. Wilson,7 David S. Zee,32 David Valle,6 David J. Amor,7,17 Melanie Bahlo,1,2,34 and Paul J. Lockhart7,17,34.

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Genomic technologies such as next-generation sequencing (NGS) are revolutionizing molecular diagnostics and clinical medicine. However, these approaches have proven inefficient at identifying pathogenic repeat expansions. We performed genetic studies of a cohort of 35 individuals from 22 families with a clinical diagnosis of cerebellar ataxia with neuropathy and bilateral vestibular areflexia syndrome (CANVAS). Analysis of whole-genome sequence (WGS) data with five independent algorithms identified a recessively inherited intronic repeat expansion [(AAGGG)exp] in the gene encoding Replication Factor C1 (RFC1). This motif, not reported in the reference sequence, localized to an Alu element and replaced the reference (AAAAG)11 short tandem repeat. Genetic analyses confirmed the pathogenic expansion in 18 of 22 CANVAS-affected families and identified a core ancestral haplotype, estimated to have arisen in Europe more than twenty-five thousand years ago. WGS of the four RFC1-negative CANVAS-affected families identified plausible variants in three, with genomic re-diagnosis of SCA3, spastic ataxia of the Charlevoix-Saguenay type, and SCA45. This study identified the genetic basis of CANVAS.

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

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

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

Searching for modifier effects of variation in Untranslated Regions (UTR) of the ataxin-3 gene (ATXN3) in Machado-Joseph disease (MJD): a pilot study

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 105

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Five (5') and three prime (3') untranslated regions (5'UTRs and 3'UTRs) provide essential elements to the transcriptional and post-transcriptional process. Characterization of the UTRs remains mostly unexplored for *ATXN3*, the Machado-Joseph disease (MJD/SCA3) causative gene. MJD is an incurable autosomal dominant polyglutamine disorder of late onset. The CAG tract size at *ATXN3* explains only partially the variance in the age at onset (AAO), suggesting the existence of genetic modifiers. We analyzed the variation in 5'UTR and 3'UTR of*ATXN3* in MJD patients and search for potential modifier effects on AAO. One hundred DNA samples from blood of MJD patients were sequenced either by Next Generation Sequencing (NGS) or Sanger sequencing. The impact of the variants was predicted by *in silico* analysis: (1) in 5'UTR, transcription factor binding sites (TFBS) and transcription factors (TFs) affinity were evaluated; (2) in 3'UTR, miRNAs binding sites, RNA binding proteins (RBPs) binding sites and polyadenylation signals were analyzed. ANCOVA was used to test the effect on AAO of variants found in 5'UTR and the impact of rs910369 in 3'UTR, previously reported as decreasing AAO onset in MJD. One variant, rs12147767 was identified in the 5'UTR. The C*allele is predicted to cause the loss of one TFBS and of affinity for three TFs. For this variant, the average AAO was similar in C*allele carrier patients compared to those with the T*allele, not supporting an effect in the AAO. Nevertheless, a tendency for a later onset in the patients with the C*allele was observed. Nine variants were found in the 3'UTR: rs709930, rs7158238, rs3092822, rs7158733, rs10151135, rs910369, rs55966267, rs11628764 and rs1055996. Two variants were predicted to have a deleterious effect, 3 variants to change miRNAs binding sites and 6 variants to impact in RBP binding sites. For the rs910369, similar AAO between A*allele and C*allele patients was observed. Therefore, findings previous reported were not replicated in the Azorean MJD cohort. The use of RNAi to silence mutant *ATXN3* has been shown to be a promising therapeutic approaches for MJD; knowledge concerning variation in the regulatory regions of this gene, namely at the 3'UTR is therefore of relevance.

Homozygous pathogenic variant in BRAT1 associated with non-progressive cerebellar ataxia

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 76

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Objective: To investigate the pathogenicity of a novel homozygous *BRAT1*variant in two siblings with non-progressive cerebellar ataxia through functional studies on primary and immortalized patient cell lines.

Methods:BRAT1 protein levels and ATM activity in patient-derived and control cell lines were assessed by western blotting. The impact of the novel *BRAT1*variants on mitochondrial function was also assessed, by comparing patient and control cell lines forrates of oxygen consumption and for phosphorylation (S293) of the E1subunit ofpyruvate dehydrogenase(PDH).

Results: Two male siblings with non-progressive cerebellar ataxia, mild intellectual disability and isolated cerebellar atrophy were found to be homozygousfor a c.185T>A (p.Val62Glu) variant in *BRAT1*by whole exome sequencing. Western blotting revealed markedlydecreased BRAT1 protein levels in lymphocytes and/or fibroblast cells from both affected siblings compared to control cell lines. There were no differences between the patient and control cells in ATM kinase activation, following ionizing radiation. Mitochondrial studies were initially suggestive of a defect in regulation of PDH activity, but there was no evidence of increased phosphorylation of the E1subunit of the PDH complex. Measurement of oxygen consumption ratessimilarly failed to identify differences between patient and control cells.

Conclusion: Bi-allelic pathogenic variants in *BRAT1*can be associated with non-progressive cerebellar ataxia, a phenotype considerably milder than previously reported. Surprisingly, despite the molecular role currently proposed for BRAT1 in ATM regulation, this disorder is unlikely to result from defective ATM kinase or mitochondrial dysfunction.

Late-onset hereditary dominant episodic ataxia in French Canadians

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 67

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The episodic ataxias (EA) are a heterogeneous group of dominantly inherited disorders. Eight different forms of EA have been described based on identified causative genetic variants or clinical features. Mutations in *KCNA1*(EA1) and *CACNA1A* (EA2) have been reported in multiple kindred and account for the majority of reported EA cases worldwide. We recruited a cohort of 65 French Canadian patients from 37 families with an undetermined EA phenotype. Mutations in known EA genes were excluded. All patients presented with recurrent attacks of ataxia that were often triggered by alcohol or physical activity, 75% of whom developed progressive ataxia. The mean age at onset was 54.4 years (range: 30-72) for the first episode, and 60 years (45-72) for the progression of ataxia. Ataxia was of mild to moderate severity with a mean SARA score of 8.9/40 (1-18). Interictal downbeat nystagmus was observed in more than 90% of patients, while diminished vibration sense was present in 50% of cases on clinical examination. Migraines were comorbid in half the patients. Acetazolamide was effective for reducing the frequency of attacks in 9% of patients. Brain MRI showed mild to moderate cerebellar atrophy in more than 50% of permanently ataxic cases. Variable expressivity in the severity of episodic attacks and ataxia progression was observed across all families. Whole exome sequencing and whole genome sequencing in the three largest families failed to reveal a shared potentially pathogenic rare genetic variant. Approximately 640,000 SNPs were genotyped in the largest family which included 8 affected and 11 unaffected members. Linkage analysis uncovered a segregated locus on chromosome 16, which was further supported by genotyping of 9 more polymorphic sequence-tagged sites markers. Copy number variation and loss of heterozygosity analyses did not reveal major rearrangements that segregated with the disease on chromosome 16. Efforts are ongoing to uncover the underlying causative mutation. In conclusion, we report a new form of late-onset progressive EA in French Canadians likely related to a locus on chromosome 16.

The Joseph and Machado lineages and their sublineages in families with Machado-Joseph disease spread worldwide

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 274

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Machado-Joseph disease (MJD; also known as spinocerebellar ataxia type 3, SCA3) is one of the neurological disorders caused by an unstable oligonucleotide repeat. Its molecular basis is a polymorphic $(CAG)_n$ tract in the *ataxin-3* gene (*ATXN3*;14q32.12), usually ranging 61-87 repeats in patients.Although MJD is the most frequent dominant

ataxia worldwide, our previous studies suggested that *de novo* expansions from the normal range would not explain its worldwide prevalence; intermediate size alleles (45-60 CAGs) are noticeably rare. To date, only two major MJD mutational origins have been identified: (1) the Machado lineage, more recent and most frequently observed in parts of Portugal; and (2) the ancient Joseph lineage of Asian origin, spread worldwide. We extended our previous studies to a larger number of markers and tested their potential for additional resolution of MJD haplotypes.

Thus, we analysed a total of 370 families, from 30 populations: Portugal (132), France (29), Spain (18), Germany (15), United Kingdom (3), The Netherlands (2), Belgium (1), Norway (1); Taiwan (28), Japan (27), China (17), Israel (6), India (4), Yemen (2), Cambodia (2), Australian aborigines (2), Thailand (1); USA (26), Brazil (24), Peru (2), West Indies (2), French Guyana (1); Mali (4), Morocco (2), Algeria (1), Ghana (1), Ivory Coast (1), Nigeria (1), Somalia (1), São Tomé and Príncipe (1); and a few of unknown origin (13) sent to us.

We assessed a set of 30 SNPs, in a 12 kb region in *cis* with the $(CAG)_n$ expansion, to establish lineages; and genotyped 7 STRs flanking *ATXN3*, to construct phylogenetic networks and compare gene diversity among populations, and age of each lineage and their sublineages.

In addition to the two major ("pure") Joseph (210) and Machado (90 families) lineages, we observed 7 sublineages, differing from the two main haplotype backgrounds at 1-3 out of 30 variant sites analysed.

The Joseph-Groote sublineage (named after the aboriginal communities of Groote Eylandt, where MJD was described in 1996), carrying a different variant in rs56268847, was present in 33 Asian families (12 from China, 10 from Japan, 6 from Taiwan, 3 from India, 1 from Cambodia, 1 from Thailand); 1 from the USA; and the 2 Australian aboriginal kindreds previously reported.

The Joseph-Ta'izz sublineage (named after the region in Yemen from where the first known Yemenite-Jewish family, described in 1994, originated) differed from the Joseph background at two SNPs (rs12895357 and rs12588287): it was observed in 15 MJD families, mostly from Africa (4 from Mali, 1 from Ghana, 1 from Ivory Coast and 1 from Morocco); 2 from Yemen; 2 from Spain; 3 from the USA; 1 from the West Indies; and the 6 Yemenite-Jewish families where this haplotype has been first reported.

Finally, the main Joseph lineage showed also a single variation at either rs12895357 or rs12588287, in 1 Japanese and in 1 USA family, respectively.

In the Machado haplotype background, a single variant in rs111735934 was present in 6 MJD families (2 from Peru, 2 from the USA, 1 from the West Indies and 1 of unknown origin). Additionally, 3 Portuguese families carried an rs12895357 variant, but all shared flanking STR haplotypes with other families with the Machado lineage, suggesting a recurrent mutation in this SNP.

Lastly, 1 family from the French Guyana showed a puzzling "mixed" haplotype, as it seemed to harbour a core of 3 SNP variants distinctive of the Joseph lineage, but flanked by a Machado background on either side.

Based on extended STR-haplotypes, the highest gene diversity was observed, as before, in Joseph lineage families from the USA (0.91±0.05) and Asia (0.88±0.03), followed by central European populations (around 0.50), with the Portuguese families showing the lowest diversity (0.18±0.08).

Contrarily to other repeat-associated diseases, MJD-causing expansions do not seem to occur frequently on multiple background haplotypes. Also, normal and expanded repeat ranges are widely separated and "intermediate-size alleles" are notoriously rare. Thus, geographic differences in MJD prevalence may probably be explained by migration routes, rather than by *de novo* pathogenic expansions. In addition to its epidemiological and historical interest, these variants could be of great value (1) to study phenotypic variation and genetic modifiers linked to these haplotypes, (2) to study genetic instability of the CAG repeat, and (3) for therapeutic interventions, including gene-based targeting, namely in the best design for allele-specific *ATXN3* silencing, in order to be applicable to most MJD patients.

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

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

Mr. Feng Wang [1]

1. *UMass Medical School*

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

Expansion of a GCA-repeat in the GLS gene is associated with progressive ataxia and developmental delay.

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 309

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Using detailed clinical and biochemical phenotyping, combined with whole-genome sequencing and the Expansion Hunter algorithm, we have identified an inborn error of metabolism caused by expansion of a GCA-repeat tract in the 5' untranslated region of the gene encoding glutaminase (*GLS*). The expansion was initially observed in three unrelated patients who presented with an early-onset delay in overall development, progressive ataxia, and elevated levels of glutamine. In addition to ataxia, one patient also showed cerebellar atrophy. Two patients were heterozygous for a large GCA-expansion and a deleterious point mutation, whilst the third had two expanded alleles. The expansion was associated with a relative deficiency of *GLS* messenger RNA produced from the expanded allele. This was accompanied by epigenetic changes, including decreased levels of acetylated H3 and H3K4me3, histone modifications characteristic of active genes. Increased levels of H3K9me3, a modification typical of repressed genes, was also observed in the patient with 2 expanded alleles. Notably, these changes were not accompanied by evidence of aberrant DNA hypermethylation.

Topic: Cellular and Animal Models of Disease

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

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

Ms. Crystal Davis [1], ***Dr. Cat Lutz*** [1], ***Dr. Laure Case*** [1], ***Ms. Emily Lowell*** [1], ***Ms. Elizabeth Rocker*** [1], ***Ms. Anna Vanasse*** [1]

1. *The Jackson Laboratory*

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

Direct neuronal reprogramming for the study of Friedreich ataxia

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

Dr. Saul Herranz-Martin [1], ***Dr. Alfredo Gimenez-Cassina*** [1], ***Prof. Javier Diaz-Nido*** [1]

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

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

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

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

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

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

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

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

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

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

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

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 181

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

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

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 178

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

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

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

Cerebellar organoids recapitulate development of the cerebellum

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 173

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Abstract

Understanding the cerebellum, which regulates motor coordination and also aspects of executive function, is vital to addressing the broad repertoire of diseases in which the cerebellum is affected. The cerebellum forms according to a highly stereotyped developmental programme into a well-organized laminar structure, containing one of the largest and most metabolically active neurons (the Purkinje neuron) as well as the most abundant neurons in the brain (the granule cell). Protracted development after birth makes it especially vulnerable to insult with overwhelming evidence for dysfunction of the cerebellum converging on the sole output neuron, the Purkinje cell, in many cerebellar diseases.

Regulatory mechanisms driving cell-fate specification have been observed in various animal models; however, studying cerebellar development in humans poses several significant challenges, including acquisition of relevant material to study. One of the means to address this is the use of pluripotent stem cells, which offer the opportunity to model human cerebellar development and disease by providing a powerful, manipulable platform.

Many of the current cerebellar differentiation protocols are reliant on co-culture with murine cerebellar neurons as monolayers and as such suffer from undefined, xenogeneic culture conditions. Furthermore, the opportunity for 2-dimensional culture settings to recapitulate the complex structural arrangement of the cerebellum is limited. Major advances in recent differentiation protocols based using organoids built through reproducing self-inductive signalling centres has provided an exciting avenue to produce more realistic cellular models. This includes modelling processes relevant to development and disease such as polarity establishment, neuronal migration and synaptic wiring/pruning.

We developed a method to derive and culture cerebellar organoids to address these restrictions by differentiating human induced pluripotent stem cells (hiPSCs) that express markers of both cerebellar germinal zones, consistent with *in vivo*cerebellar development as structurally intact 3D entities. As a means to investigate whether these organoids truly recapitulate development of the human cerebellum, and furthermore what stage of maturity they can reach, we employed single-cell sequencing which revealed transcriptionally-discrete populations encompassing the major cerebellar neuronal cell types including proliferative/migratory granule cells, roof plate, choroid plexus, Bergmann glia, Purkinje cells and Deep Cerebellar Nuclei. Importantly, variability between organoids was minimal with robust representation of all identified cerebellar populations. Further, we show the contribution of basement membrane signalling to influencing both cellular composition of the organoids, and also developmentally-relevant gene expression programs that are active in specific cerebellar populations. This model system has exciting implications for studying cerebellar development and disease most notably by providing a xeno-free conditions that provides a more biologically relevant culture setting.

A patient-derived 3D cerebellar model for spinocerebellar ataxia type 1

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 163

Dr. Willeke van Roon-Mom [1], ***Dr. Ronald Buijsen*** [1]

1. *Leiden University Medical Center*

Introduction

Spinocerebellar ataxia type 1 (SCA1) is a hereditary neurodegenerative disease caused by a polyglutamine expansion in the ataxin-1 protein that results in aberrant protein aggregation and neuropathology, mainly in the cerebellum. Although, several SCA1 mouse models have been generated that recapitulate prominent aspects of the human disease there are considerable developmental, genetic and physiological differences between humans and mice. The aim of our study is to generate a patient-derived cellular model of SCA1 that will more faithfully reflect the human specific aspects of the disease.

Methods

Skin biopsies were obtained from four SCA1 patients and four related healthy controls. Control and disease-specific hiPSC lines were generated using the integration-free Sendai virus based method. On day 0, iPSCs were dissociated and cells were resuspended in induction medium containing factors selected to mimic the self-inductive properties of the isthmic organiser. Recombinant fibroblast growth factor 2 (FGF2) was added to the culture on day 2. On day 21, cell aggregates were incubated for 14 days in differentiation medium, consisting of neurobasal medium supplemented with GlutaMAX and N2. Cell aggregates were harvested for immunocytochemistry and QPCR at days 21 and 35 of differentiation.

Results

We generated patient-derived hiPSC lines that are SeVfree, express pluripotency markers, displayed a normal karyotype, retained the mutation (length of the CAG repeat expansion in the *ATXN1* gene) and were able to differentiate into the three germ layers *in vitro*. Furthermore, we differentiated these cells into cerebellar progenitors that express hindbrain-specific transcription factors, Purkinje cell precursor markers, and cerebellar markers (immunocytochemistry and QPCR).

Conclusion

We successfully generated cerebellar progenitor cultures that are useful to study SCA1 disease mechanisms and therapeutic intervention strategies.

Role of astrocytes in Friedreich's Ataxia neurodegeneration

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 151

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

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

In this study, we are first characterizing a model of FA-astrocytes, either under resting or inflammatory conditions. To this purpose, primary rat cerebellar astrocytes were transfected for 72 h with specific FXN siRNA and subjected or not to conditions mimicking inflammation, i.e. 24 h-treatment with a mix of cytokines (CKs; IL-1β, TNFα and INFɣ). Both FXN transcript level and protein expression were significantly reduced (up to 30% of WT cells) by siRNA transfection and CK treatment did not alter these effects. FA-astrocytes showed the typical cellular hallmarks of FA, with reduced mitochondrial membrane potential and increased levels of oxidative stress, when compared to control cells. On the other hand, FA-astrocytes appeared activated by CK treatments similarly to control cells, with comparable up-regulation of inducible nitric oxide synthase (iNOS) and the consequent release of nitric oxide. Other markers of activation as well as a panel of several cytokines are under investigation, in order to evaluate whether the activation process might be altered in FA astrocytes, with detrimental effects towards the neurons.

Since we aim to understand the role of astrocytes on neurodegeneration in FA, we are also investigating the effects of conditioned medium collected from FA- or control- astrocytes, at rest or activated, on cerebellar granule neurons (CGNs). It appears that the conditions medium from CK-activated FA-astrocytes promoted a significant increase of neuronal death.

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

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

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

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

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

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

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

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

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

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

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

consistent with levels found in FRDA patients.

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

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

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 96

Mr. Luca Panarello [1], ***Dr. Giulia Alfedi*** [2], ***Dr. Giorgia Alaimo*** [1], ***Dr. Riccardo Luffarelli*** [2], ***Dr. Ivano Condò*** [1], ***Dr. Monica Benini*** [2], ***Prof. Florence Malisan*** [1], ***Prof. Roberto Testi*** [2], ***Dr. Alessandra Rufini*** [2]

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

We then used the obtained cardiomyocytes to test the effect of etravirine on this cell type. Our results show that etravirine promotes a statistically significant 40% increase in the levels of mature frataxin after 24 hours of treatment. In the future it will be important to functionally characterize these cells and highlight phenotypic differences between healthy and FRDA cardiomyocytes. In particular, we will evaluate sensitivity to oxidative stress-mediated cell death and the iron handling capability using the Calcein-AM quenching assay after exogenous iron overload.

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Defining the Frataxin G130V pathogenic mechanism in Friedreich's ataxia

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 83

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

SCA1-specific phenotypes in patient-derived neurons

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 56

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Introduction

Spinocerebellar ataxia type 1 (SCA1) is a hereditary neurodegenerative disease caused by a polyglutamine expansion in the ataxin-1 protein (Orr, Chung et al. 1993) that results in aberrant protein aggregation and neuropathology, mainly in the cerebellum (Seidel, Siswanto et al. 2012). Furthermore, it has been shown that the ataxin-1 protein plays a role in neurodevelopment (Edamakanti, Do et al. 2018) and the regulation of bioenergetics and metabolic alterations in the cerebellum of SCA1 mouse models (Sanchez, Balague et al. 2016).

The aim of our study is to (1) generate patient-specific models and (2) determine a SCA1-specific phenotype in these models.

Methods

Skin biopsies were obtained from four SCA1 patients and four related healthy controls and human induced Pluripotent Stem Cell (hiPSC) lines were generated using the integration-free Sendai virus based method (Buijsen, Gardiner et al. 2018). The presence of aggregates was assessed using immunohistochemistry while dendritic length and number of branching points were evaluated after transient GFP transfection. Finally, the two major energy pathways of the cell, mitochondrial respiration and glycolysis, were measured using the Seahorse XF96 Extracellular Flux Analyzer where oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) respectively were used as outcome measures.

Results

We successfully generated patient-derived fibroblast and hiPSC lines. After differentiation of the SCA1 hiPSCs into neuronal cells, ataxin-1 positive aggregates were present. Furthermore, there was a reduction in cell branching and branch-length indicating neurodevelopmental delay. A deficit in bioenergetics was shown by a decreased maximal respiration and an increased glycolysis in patient-derived cells compared to control cells, supporting a role for mitochondrial dysfunction in SCA1 pathogenesis.

Conclusion

We have generated an hiPSC□based model for SCA1 that recapitulates key pathological features of this disease. Moreover, our hiPSC□based model provides a valuable tool to investigate the pathogenic mechanisms of SCA1 and to screen for drugs that may prevent or rescue neurodegeneration in SCA1.

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

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 82

Ms. Maria Russi [1], ***Dr. Elodie Martin*** [1], ***Dr. Hervé Tricoire*** [1], ***Dr. Veronique Monnier*** [1]

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

CAG-induced blood-brain barrier dysfunction in an isogenic iPSC model of juvenile-onset Huntington's disease

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 310

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The human blood-brain barrier (BBB) is comprised of brain microvascular endothelial cells (BMECs), pericytes, and astrocytes, which critically maintain neuronal homeostasis. BBB dysfunction has been implicated in the pathogenesis of Huntington's disease (HD) [1]. HD is caused by expansion of cytosine–adenine–guanine (CAG) repeats in the huntingtin gene. Mutant huntingtin (mHTT) protein, produced by neurons and astrocytes, aggregates within the brain parenchyma and BBB. However, the direct role of BMECs in this process remains unclear. Recent work suggests that BMECs of HD patients are autonomously dysfunctional; human induced pluripotent stem cells (hiPSCs) differentiated into BMECs from HD patients exhibit elevated angiogenic potential and deficient BBB phenotype compared to healthy cell lines [2].

To further elucidate mechanisms of BBB dysfunction we utilize an isogenic pair of hiPSCs: one line is derived from a juvenile HD patient with 180 CAG repeats in the huntingtin gene, while the other is CRISPR-corrected [3]. These cells were previously used to show CRISPR-correction of phenotypic abnormalities of iPSC-derived neurons. Here, we explore phenotypic abnormalities of iPSC-derived BMECs. We find that expanded CAG repeats reduce the transendothelial electrical resistance of BMECs (~3-fold), corresponding with mislocalization of tight junction protein expression. Additionally, expanded CAG repeats impart elevated susceptibility to oxidative damage, as measured by reductions in electrical resistance when perturbed with hydrogen peroxide. Our results suggest that BMECs directly contribute to loss of BBB function during HD, providing a novel target for therapeutic strategies.

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Topic: Natural History, Biomarkers and Endpoints

polyQ-ATXN3 proteins in biofluids, a biomarker for SCA3

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 304

Dr. Mercedes Prudencio [1], ***Dr. Rana Hanna AL-Shaikh*** [2], ***Mrs. Karen Jansen-West*** [1], ***Dr. William Freeman*** [2], ***Dr. J van Gerpen*** [2], ***Dr. Eric Eggenberger*** [2], ***Dr. Mark LeDoux*** [3], ***Dr. Ronald Pfeiffer*** [4], ***Dr. John Fryer*** [1], ***Dr. Zbigniew Wszolek*** [2], ***Dr. Leonard Petrucelli*** [1]

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Spinocerebellar ataxia 3 (SCA3), the most common spinocerebellar ataxia worldwide, is characterized by the accumulation of polyglutamine (polyQ) protein aggregates derived from translation of the CAG trinucleotide expansions in ataxin 3 (ATXN3). No effective treatment exists to stop or ameliorate this devastating and progressive movement disorder. The main focus for therapeutic intervention in SCA3 is to reduce the levels of mutant ATXN3. In order to monitor drug efficacy in human trials it is imperative to have access to proper means of tracking disease progression. In addition, besides genetic testing, there are no means to determine whether and when a given person may develop SCA3. While no validated biomarker of this kind yet exists, polyQ-ATXN3 proteins may be most suitable to fill this urgent need. We recently developed immunoassays to measure polyQ and total ATXN3 proteins in human biofluids. Our exciting preliminary data demonstrate that polyQ-ATXN3 proteins significantly accumulate in plasma and CSF of SCA3 patients compared to controls. Confirmation that polyQ-ATXN3 proteins are measureable in these biofluids, would greatly facilitate identification of SCA3 subjects in the course of standard diagnostic workups. Furthermore, demonstrating that polyQ-ATXN3 protein levels are associated with rate of disease progression and severity of phenotype would be a significant advance, with potential to serve as an outcome measure in the evaluation of experimental treatments. Overall, our proposed studies are anticipated to have significant impact in the field, and contribute to efforts to advance therapy development and estimation of prognosis for SCA3.

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

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 302

Dr. Gilbert Thomas-Black [1], ***Dr. Christian Rummey*** [2], ***Dr. David Lynch*** [3], ***Prof. Paola Giunti*** [4]

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

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

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

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

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

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

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

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

log pm/ml vs 2.48 log pm/ml, p = 0.227)
In the overall data, when stepwise increasing the threshold for age, subgroup analyses show that at ages > 35, the group difference is still elevated in FRDA, but not statistically significant anymore (2.63 vs 2.45 log pm/ml, p = 0.077).
Conclusion – Our results indicate that Nf-L as a biomarker in FRDA might be highly useful, especially in the early AAO group.

Onset symptoms and time to diagnosis in Friedreich´s Ataxia

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 301

Dr. Elisabetta Indelicato [1], ***Dr. Wolfgang Nachbauer*** [1], ***Dr. Andreas Eigentler*** [1], ***Dr. Matthias Amprosi*** [1], ***Prof. Paola Giunti*** [2], ***Dr. Caterina Mariotti*** [3], ***Dr. Javier Arpa*** [4], ***Prof. Alexandra Durr*** [5], ***Prof. Thomas Klopstock*** [6], ***Prof. Ludger Schöls*** [7], ***Prof. Thomas Klockgether*** [8], ***Dr. Katrin Bürk*** [9], ***Prof. Massimo Pandolfo*** [10], ***Prof. Jörg Schulz*** [11], ***Dr. Sylvia Boesch*** [1]

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

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

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

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

Multimodal imaging to unravel structural abnormalities underlying dementia and mild cognitive impairment in Spinocerebellar ataxia 2

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 293

Dr. Albert Stezin Sunny [1], ***Mr. Sujas Bhardwaj*** [1], ***Mr. Sunil Khokhar*** [1], ***Dr. Sanjeev Jain*** [1], ***Dr. Rose Dawn Bharath*** [1], ***Dr. Shantala Hegde*** [1], ***Dr. Jitender Saini*** [1], ***Dr. Pramod Kumar Pal*** [1]

1. *National Institute of Mental Health and Neurosciences (NIMHANS)*

Introduction

Cognitive impairment in Spinocerebellar ataxia 2 (SCA2) is estimated to have a prevalence of 5 - 42%. However, due to the rarity of the disorder, the cognitive profile of SCA2 is poorly characterized and their structural underpinning poorly understood. In this study, we systemically evaluated the cognitive functions in patients with SCA2 and classified them on the basis of severity of disease into SCA2 with dementia (SCA2-D), mild cognitive impairment (SCA2-MCI), and no cognitive impairment (SCA2-NCI).

Methodology

Neuropsychological assessment: Thirty-three subjects with genetically proven SCA2 and 30 age, gender and education matched- healthy controls underwent detailed neuropsychological evaluation. Five cognitive domains were evaluated- complex attention (colour trails-1, digit vigilance, serial subtraction test), executive function (color trails-2, stroop test, spatial span, Wisconsin card sorting test, tower of London, NBACK test), language (controlled oral word association, animal naming test), learning and memory (Immediate and delayed recall from complex figure test and auditory verbal learning test), perceptual-motor function (copy score of complex figure test and pentagon drawing test). Only time-independent subscores were used for this study to remove confounding effects of motor dysfunction.

Factor analysis was performed to reduce the number of dimensions in each cognitive domain to two-factor subscores which were then converted to z-scores. A score below two standard deviations from the mean value was regarded as impaired. Patients with impairment in two sub-score of the same domain or one impaired score each in two separate domains were classified as SCA2-MCI. If the cognitive dysfunction was more severe than SCA2-MCI, the patient was regarded as SCA2-D.

Neuroimaging: MRI scans of patients with SCA2-D, SCA2-MCI and SCA2-NCI were performed in a 3-Tesla Siemens Skyra MRI. T1 structural and diffusion sequences were used to perform Voxel-based morphometry (VBM) and Diffusion tensor imaging- Tract based spatial statistics (DTI-TBSS) to identify the grey matter (GM) and white matter (WM) microstructural abnormalities, respectively. VBM was performed using Matlab based- SPM12 and CAT12 software. DTI-TBSS was performed using FSL software in a Unix environment. Comparisons were performed between (i) SCA2 with cognitive impairment (SCA2-D + SCA2-MCI) versus SCA2 without cognitive impairment (ii) SCA2-D versus SCA2-MCI. A statistical threshold of $p<0.05$ (FWE corrected) was considered to be significant.

Results

There were no significant differences in the demographic and clinical scores of (i) patients with and without cognitive impairment and (ii) with SCA2-D and SCA2-MCI. We identified cognitive impairment to be present in 75.7% (n =25) patients. Among them, 54.5% (n =13) had SCA2-D; 21.2% (n =5) had SCA2-MCI and 24.2% (n=6) did not have any cognitive impairment. The SCA2-dementia and SCA2-MCI groups were different only with respect to the higher prevalence of complex attention and perceptual-motor impairment in the SCA2-D group.

Compared to patients without cognitive dysfunction, SCA2 with cognitive impairment had significantly more GM atrophy of the cerebellum (culmen, declive, quandrangular lobule, and simple lobule of both sides and left cerebellar tonsil), right primary motor cortex, bilateral sensorimotor cortex, and left superior frontal gyrus (Table 1). Patients with SCA2-D had significantly more GM atrophy of the precuneus and premotor cortex, whereas patients with SCA2-MCI had more of posterior cingulate and middle temporal gyrus atrophy. Compared to SCA2-MCI, SCA2-D had significantly more GM atrophy of the angular gyrus. There were no areas of GM atrophy in SCA2-MCI compared to SCA2-D.

There were no significant WM changes (FA, MD, AD, RD) observed in SCA2 with cognitive impairment compared to SCA2-NCI. Similarly, there were no significant WM microstructural changes of comparison of SCA2-D and SCA2-MCI.

Conclusion

Cognitive impairment in SCA2 is a result of damage to the cerebellum and extra-cerebellar regions, predominantly the frontal cortex. SCA2-D and SCA2-MCI were different only in terms of GM atrophy of the angular gyrus. The absence of any significant WM changes suggests that cognitive impairment is mainly due to changes affecting the cell bodies of the neurons in the GM. Hence, MRI changes could be used as biomarkers for the development of cognitive impairment in SCA2. Our results also provide evidence for the higher prevalence of cognitive impairment and the presence of dementia and MCI in SCA2.

Mass spectrometric characterization of the cerebrospinal fluid proteome to identify potential biomarkers for spinocerebellar ataxia 2

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 288

Dr. Albert Stezin Sunny [1], ***Dr. Akshada Gajbhiye*** [2], ***Dr. Gajanan Sathe*** [2], ***Dr. Shantala Hegde*** [1], ***Dr. Jitender Saini*** [1], ***Dr. Rose Dawn Bharath*** [1], ***Dr. Faruq Mohammed*** [3], ***Dr. Meera Purushottam*** [1], ***Dr. Sanjeev Jain*** [1], ***Dr. Pramod Kumar Pal*** [1], ***Dr. Akhilesh PANDEY*** [4]

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Introduction:

Spinocerebellar ataxia 2 (SCA2) is an autosomal dominant SCA characterized by the presence of cerebellar ataxia, slow saccadic eye movements, and peripheral neuropathy. At present, disease severity and progression are evaluated clinically using semi-quantitative scales. However, these measures are time consuming, often subjective and have only moderate to fair psychometric properties. In this study, we attempted to identify objective biomarkers by characterizing the CSF proteome of patients with genetically proven SCA2.

Methodology:

We used CSF from 21 patients with SCA2 and 19 patients without SCA2 (i.e. normal pressure hydrocephalus, non-specific headache, idiopathic intracranial hypertension, and subjects undergoing spinal anesthesia) using lumbar puncture. All patients were examined using the Schedule for assessment and rating of ataxia (SARA) scale and SCA functional index (SCAFI) to quantify the severity of ataxia and functional severity.

In the discovery phase of the experiment, we used 5 samples from each group and compared the the CSF proteomes using high□resolution mass spectrometry and tandem mass tag (TMT)-based multiplexing technology. Only proteins with a minimum of 1.5 fold change compared to controls were considered as significantly altered proteins. These proteins were then shortlisted for the validation phase of the experiment on the basis of function, expression, localization, and association with other neurological disorders. The shortlisted 'potential biomarkers' were then validated using targeted parallel reaction monitoring (PRM) assays.

Results:

A total of 673 proteins was identified in the CSF of patients with SCA2 of which 109 proteins were significantly altered in abundance. Among these proteins, 94 proteins were upregulated and 15 were downregulated. Based on the function, expression, and localization of these proteins, we narrowed down the list of proteins for validation to 31 proteins. Targeted PRM assays of individual samples led to identification of 19 proteins as elevated in all samples. These included ATP6AP1, CDH13, CLSTN1, CHL1, CNTN1, DAG1, IGFBP2, NPTX1, NEGR1, NELL2, NRCAM, NCAN, NELL2, NPTXR, PKM, PCSK1N, PENK, SCG5, and SCG3. Some of these proteins including NPTX1, DAG1, PENK, and SCG5 exhibited significant correlation with clinical and functional severity of the disease. These proteins also have specific roles in cerebellar, cortical and subcortical function.

Conclusion:

The proteins that are found to be altered in the CSF of patients with SCA2 could be used for diagnosis, monitoring disease progression and therapeutic response once they are validated in larger studies.

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

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 286

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

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

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

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

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

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

Quality of Life displays changes in preclinical phases of Machado-Joseph Disease/Spinocerebellar Ataxia type 3 (BIGPRO Study)

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 285

Ms. Gabriela Bolzan [1], ***Ms. Camila Oliveira*** [1], ***Ms. Gabriela Ecco*** [1], ***Ms. Amanda Henz*** [1], ***Ms. Anastacia Rocha*** [1], ***Ms. Nathalia Kersting*** [1], ***Mrs. Mariana Rieck*** [1], ***Ms. Ana Carolina Martins*** [1], ***Prof. Vanessa Bielefeldt Leotti*** [2], ***Prof. Maria Luiza Saraiva-Pereira*** [3], ***Prof. Laura Jardim*** [4]

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Background and Objective:

Spinocerebellar Ataxia type 3/Machado-Joseph Disease (SCA3/MJD) is an autosomal dominant neurodegenerative disorder that plays with a constellation of motor and non-motor symptoms, among which ataxia stands as the most prominent one. This disease progresses inexorably and no current treatment is available. Quality of life has been increasingly valued as an outcome in clinical research for its importance in displaying the patient's perspective over illnesses and healing. Changes in quality of life have been previously reported in symptomatic phases of SCA3/MJD. We here report baseline results from Quality of Life assessments in a longitudinal study investigating symptomatic and asymptomatic SCA3/MJD carriers (BIGPRO Study - bigpro.webnode.com).

Methods:

Between August 2017 and November 2018, subjects who were symptomatic or at 50% risk for SCA3/MJD filled the self-administered questionnaires EQ-5D-3L and SF-36. Five dimensions were evaluated by EQ-5D, each of them in 3 levels. EQ-5D Index was calculated based on the Argentinian value-set (doi: 10.1111/j.1524-4733.2008.00468.x). For SF-36, raw scores were calculated according to developer's instructions. Clinical scales NESSCA, SARA, ICARS, INAScount, CCFS and SCAFI were obtained. Subjects at risk were double-blindly genotyped. Age at onset of gait ataxia was recorded, and the time that had passed by since the onset of the disease was calculated (time after onset). For asymptomatic carriers (SARA < 3), Time to Onset was calculated using the CAG repeat length at the expanded allele (CAGexp) to estimate the average time left until the onset of gait ataxia (doi: 10.1111/ene.13779). Asymptomatic carriers were classified according to the predicted age at onset at birth and divided into two groups: asymptomatics far from age of onset (AFF) – when predicted to start symptoms in more than 4 years – or near (AN) age of onset – when disease start predicted to happen in 4 or less years. TtoAfterOnset combined data on estimated time to and after onset from symptomatic and asymptomatic subjects. Statistical analysis was performed using SPSS v.19.0, and $p<0.05$ was considered as statistically significant.

Results:

Eighty-nine subjects – 30 symptomatic and 59 at 50% risk for SCA3/MJD were included (Table 1).

Four EQ-5D dimensions showed significant differences between groups - all of them related to worse scores in the symptomatic group. Although non-significant, there were stepwise changes in these four dimensions from controls to asymptomatic and then to symptomatic groups. Similarly, the symptomatic group differed from the asymp-

tomatic and the control groups for EQ-5D Index and EQ-5D Visual Analogue Scale (EQ-VAS) (Table 2). Four SF-36 domains showed differences between symptomatic subjects and controls, three of which also detected differences between symptomatics and AFF, but not between symptomatics and AN (Table 2). Among symptomatics, EQ-VAS displayed correlations with all clinical scales, having the strongest correlation appeared with NESSCA (rho=-0.612; $p<0.01$). SF-36 Physical Functioning correlated to all, except NESSCA, and the strongest correlation was found with SCAFI (rho=0.765; $p<0.001$). CCFS and SCAFI were correlated to SF-36 Pain in symptomatics (rho=-0.559, $p<0.01$; rho=0.654; $p<0.01$) and were the only scales to correlate to quality of life parameters in AN.

Discussion:

In symptomatics, different domains of quality of life were correlated to each clinical scale, suggesting that each scale might be evaluating distinct aspects contributing to patient's perception of life. The correlation of EQ-VAS with all clinical scales suggests that they do reflect important outcomes for patients, at least to some extent.

Early symptomatic stages of SCA3/MJD were associated with poorer Quality of life, when compared to controls. Although no association was seen between the AN and EQ-5D domains and no differences were found between most AN and controls' scores for SF-36, the observed replies suggest a continuum of worsening from AFF to symptomatics. This trend suggests that, even before the disease onset, carriers might already have some loss of quality of life.

Acknowledgements: CAPES, CNPq, FAPERGS, FIPE-HCPA.

Eye movements measured by video-oculography as biomarkers of pre-clinical stages in Machado-Joseph disease/Spinocerebellar ataxia type 3 (BIGPRO Study)

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 268

Ms. Camila Oliveira [1], ***Ms. Gabriela Bolzan*** [1], ***Ms. Gabriela Ecco*** [1], ***Ms. Amanda Henz*** [1], ***Ms. Anastacia Rocha*** [1], ***Ms. Nathalia Kersting*** [1], ***Mrs. Mariana Rieck*** [2], ***Ms. Ana Carolina Martins*** [1], ***Prof. Vanessa Bielefeldt Leotti*** [2], ***Prof. Maria Luiza Saraiva-Pereira*** [2], ***Prof. Laura Jardim*** [2]

1. *UFRGS,* ***2.*** *HCPA & UFRGS*

Background and Objective

Spinocerebellar Ataxia Type 3/Machado-Joseph Disease (SCA3/MJD) is an autosomal dominant disorder caused by a CAG repeat expansion (CAGexp) at the ATXN3. Causal treatment is not available yet. During recent years, promising progress has been made in the understanding of the pathogenesis. Considering that a causal therapy will probably be more efficient if started early in life, reliable biomarkers for pre-clinical stages are needed. BIGPRO (Biomarkers and genetic modifiers in a study of presymptomatic and symptomatic SCA3/MJD carriers) is a longitudinal study aiming to validate biomarkers for disease progression in SCA3/MJD since pre-clinical periods (bigpro.webnode.com). We report baseline findings obtained from eye movements measured by video-oculography from the 95 first individual participants.

Methods

Recruitment occurred between August 2017 and November 2018. Baseline data on clinical scales and oculomotor neurophysiology were collected from 95 subjects – 36 symptomatic and 59 at 50% risk for SCA3/MJD. Age at onset (AO) was considered the age at which the subject and her/his relatives first noticed gait ataxia. Time after onset was considered the time elapsed since the AO for each symptomatic subject. Genetic tests performed in at risk subjects were double-blind. For presymptomatic carriers (SARA < 3), the average time left until the onset of gait ataxia was called “time to onset”. The CAGexp was used to estimate time to onset both at birth and corrected by age, as described elsewhere (doi: 10.1111/ene.13779). Presymptomatic subjects were classified according to the predicted age at onset at birth and divided into two groups: asymptomatics far from age of onset (AFF) – when predicted to start symptoms in more than 4 years – or near (AN) age of onset – when disease start predicted to happen in 4 or less years. Time to/time after onset (TtoAfterOnset) was a unique dimension of time versus start of gait ataxia, estimated to all SCA3/MJD carriers.

Clinical outcome assessments (COAs) of interest for this report were parameters of three different domains of eye movements: saccades, pursuit and fixation, measured by video-oculography (EyeSeeCam - doi: 10.3233/VES-160579). The parameters chosen for comparisons were average reflex vertical saccade velocity (RVSV), horizontal and vertical pursuit gains, slow-phase velocity of central (SPV-C) and gaze-evoked nystagmus (SPV-GE). Parametric tests were performed when quantitative variables showed normal distributions. The statistical analysis program SPSS v.19.0 was used and results were considered statistically significant when $p<0.05$.

Results:

Thirty-seven of the 59 at 50% risk subjects were in fact SCA3/MJD carriers: 13 of them were at four or less years from their predicted AO. Overall characteristics of the 95 subjects, classified in four groups (symptomatic carriers, AN, AFF and related controls) were shown in Table 1.

All parameters under study – RVSV, horizontal and vertical pursuit, SPV-C and SPV-GE – showed statistically signif-

icant differences when the four groups were studied. However, only RVSV and SPV-GE results showed significant differences between controls and AN (**Table 2**).

TtoAfterOnset obtained from all 73 carriers of CAGexp correlated strongly with SPV-GE (rho=0.715, p<0.001) and moderately with RVSV (rho=-0.524, p<0.001) and horizontal pursuit (rho=-0.540, p<0.001). When considering only asymptomatic carriers, time to onset correlated with SPV-GE (rho=0.452, p=0.007), RVSV (rho=-0.419, p=0.014) and horizontal pursuit gain (rho=-0.411, p=0.016). As for external validity, the clinical SARA correlated better with SPV-GE (rho=0.737, p<0.001) and RVSV (rho=-0.612, p<0.001), but also with SPV-C (rho=0.364, p<0.001), horizontal (rho=-0.568, p<0.001) and vertical (rho=-0.335, p=0.001) pursuit gains.

Discussion

RVSV and SPV-GE distinguished pre-clinical carriers near the predicted age of onset and correlated well with time to onset when considering only pre-symptomatic carriers. In addition, both parameters demonstrated external validity as demonstrated with the correlation with the already validated clinical SARA. These results suggest that RVSV and SPV-GE could be good candidate biomarkers for the pre-symptomatic period in SCA3/MJD. Longitudinal observations will deepen these observations and perhaps confirm these findings.

Continuous glucose monitoring in adults with Friedreich's Ataxia

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

Dr. Jaclyn Tamaroff [1], ***Ms. Nithya Mitta*** [1], ***Ms. Sara Nguyen*** [1], ***Dr. Neil Wilson*** [2], ***Dr. Darko Stefanovski*** [2], ***Ms. Gayatri Schur*** [2], ***Ms. Anna Dedio*** [1], ***Ms. Kristin Wade*** [1], ***Dr. David Lynch*** [1], ***Dr. Ravinder Reddy*** [2], ***Dr. Andrea Kelly*** [1], ***Dr. Michael Rickels*** [2], ***Dr. Shana McCormack*** [1]

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

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

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

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

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

Results: Individuals with FA [n=9; 5 male, median age 24y (IQI 23, 36); median BMI 26.9 kg/m2 (IQI, 25.6, 29.3)] and healthy volunteers [n=16; 8 male, median age 31y (IQI 27, 43); median BMI 23.9 kg/m2 (IQI, 21.3, 26.3)] completed CGM. Individuals with FA had a median HbA1c of 5.0% (vs. 5.1% in controls), fasting glucose of 91 mg/dL (vs. 84 in controls). None of these differences was statistically significant. Median WBISI was 2.8 in in FA (vs. 4.6 in controls, p=0.019 for difference).

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

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

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

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

CITATIONS

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

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

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Introduction

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

Methods

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

Results

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

Conclusion

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

Understanding the Diagnostic Experience of Individuals with Friedreich's Ataxia

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

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

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

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

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

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

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

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

Functional mobility in autosomal recessive spastic ataxia of Charlevoix-Saguenay; a 6-year longitudinal study

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 241

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Background

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is an early onset neurodegenerative cerebellar disease. It features a triad of cerebellar, pyramidal and neuropathic involvements. Symptoms highly vary between individuals, but they usually become wheelchair-bound by the age of 35-40 years old. ARSACS strongly influence mobility, which is essential for independence in daily living activities and social activities. As for now, we know little about the natural history of ARSACS in regard to mobility issues and this could be impeding clinical trial readiness and good clinical management.

Objectives

This study aimed to: (1) document the natural history of ARSACS in regards to functional mobility over a 6-year period and (2) explore factors that may contribute to the progression of mobility limitations.

Methods

This is an exploratory longitudinal study. Data were collected at three times during the 6-year period (T1:2012-13 N=28, T2:2015-16 N=46 & T3:2017-18 N=52). For each data collection time, patients underwent 2 assessment periods of 3 hours. Over all the data collected, we used the 10 meter walking speed test to assess walking speed, the Berg Balance Scale for balance, the 6-minute walking test for walking endurance and the Timed-up and Go for overall functional mobility. Statistical analyses were done in two steps. First, descriptive statistics and Friedman tests were used to compare means between the 16 participants who did all three phases. Secondly, regressions were made using repeated mixed models for all participants who did at least two measurement times. Measurement time, age and sex were identified as covariates.

Results

Out of the 16 participants from the three phases, 2 were already constant wheelchair users and 4 lost the ability to walk even with walking aid. As expected, as the disease progresses, balance and walking distance were significantly lower for the 16 participants. Walking speed did improve a little and may be biased by the very small sample size (N=11) and introduction of walking aids that help to improve balance.

As for regressions analysis of all participants, age influenced balance (β=-1.250 (SE; 0.186)) and walking speed (β=-0.034 (SE; 0.008). But when included in the model, balance was the only factor influencing walking speed (β=0.0196 (SE; 0.0023)). Walking distance was affected by sex and age, which is expected since predicted values are computed with those two variables. When balance was included in the model for walking distance, only balance and sex were significant (β= 5.95 (SE; 0.61)) with a a mean difference of 46.51m (SE; 13.75) in favour of men. There seems to be a threshold for walking aids as the means walking speed with a walking aid for all three phases were lower than 1m/s.

Conclusion

Expanding the knowledge about natural history of a rare disease is a key point in trial readiness. It also helps clinicians to provide better interventions and to optimize clinical management of patients. In view of trial readiness,

balance and walking speed seem to be the best indicators. Berg balance scale and 10mWT are fast, easy and reliable tests in this population and could be used for clinical trials.
Results found in this study can help clinicians to optimize their interventions as they now have regression equation to compare patients to expected results for balance and walking speed. Models used in this study need validation with larger sample size in order to improve accuracy.

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

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 234

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

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

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

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

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

- Cervical spinal cord cross sectional area (CSA) is significantly decreased in FRDA patients compared to age and sex matched controls (control 84.9±03.32mm², FRDA 58.9±5.33, $\rho<0.0001$, Mann-Whitney U test). Further to this, we investigated the correlation between cord CSA and the general peripapillary retinal nerve fibre layer thickness in FRDA patients and this was found to be significant $\rho=0.491$, $\rho=0.024$ (Spearman's rho).

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

- The DTI metrics fractional anisotropy (control 0.72±0.038mm²/s FRDA 0.62±0.034 =0.0002) is significantly different between controls and patients. We have further delineated the exact areas of maximal change within the cord using the Spinal Cord Toolbox software and demonstrate that the dorsal columns display the largest degree of separation, followed by all white matter, then lateral columns then grey matter.
- Peripapillary retinal nerve fibre layer thickness correlates with GAA1 size, age at onset and SARA score as well as frataxin protein levels.

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

Motor Evoked Potential Input Output Measures and FRDA Disease Burden

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 233

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

We performed single pulse TMS in 32 individuals with FRDA using a Bistim 200[2] with a 70 mm figure-of-eight coil (Magstim, UK) paired with a frameless stereotaxy system (Brainsight, Montreal, CA) to ensure reliability and precision of stimulation. MEPs were measured with adhesive surface electrodes over the first dorsal interosseous of the dominant hand using recording hardware (CED 1902 and 1401, CED, UK) and Signal 3.13 (CED, UK). After identification of an optimal location to reliably produce MEPs, resting motor threshold was determined using established standards. In patients with RMT greater than machine output, active motor threshold was measured during compression of a small length of plastic tubing to produce a constant contraction. Twenty stimuli at 110% of MT and 120% of MT were recorded while the test subject was engaged in a mental task designed to lessen anticipation of pulses. MEP amplitude was measured as the greatest peak to peak distance for each trial, and all trials not affected by excessive movement or myogenic artifact were averaged to produce a mean MEP at each intensity. The difference in amplitudes obtained with stimulation at 110% MT and 120% MT was used as an input-output measure. The correlation of MEP amplitude difference and age, age at symptom onset, shortest GAA repeat length, product of GAA repeat length and symptom duration (a measure of disease burden), and FARS scores was evaluated by Pearson correlations, and effect of time on MEP amplitude, and amplitude difference was evaluated by repeated measures ANOVA.
Results:
The cohort included 15 females (47%), and mean age was 18 years (range 7-34). Mean age at symptom onset was 17.2 years (range 2-21). Mean length of the shortest GAA repeat was 734.5 (range 400-1150). Mean FARS score was 58.7 (range 32.5-86.5). Correlations between MEP amplitude at a single stimulation intensity (110% MT or 120% MT) and patient characteristics was modest (R = 0.22-0.43), but the correlation between MEP amplitude difference and disease burden was strong (R=0.657). Figure 1 illustrates this association across subjects, separated into patients

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

Conclusions:

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

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

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

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

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

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

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

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

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

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

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

Results and discussion:

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

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

Conclusion:

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

References:

1 – Wolz R, et al. Neuroimage 2010 Jan 15; 49(2): 1316–1325.

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

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

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Introduction

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

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

Methods

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

Results

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

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

Conclusion

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

Patient Reported Outcome Measure for Ataxia (PROM-Ataxia). Development and Validation of a patient-based assessment of ataxia symptoms and impact.

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 199

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Introduction:

Cerebellar disorders affect motor control, cognition and emotion, degrading quality of life. In neurodegenerative ataxias manifestations evolve over time. A critical gap in the management of ataxia is absence of a measure of patient experience, needed for assessment of natural history and subjective response to interventions. We developed and validated the PROM-Ataxia derived from patients reports of their symptoms and affected activities.

Methods:

Patient input was recruited in two phases following guidelines of the Patient-Reported Outcomes Measurement Information System.

A semi-structured survey was administered to cerebellar ataxia patients to assess the conceptual framework of symptoms and reports of affected activities / abilities. It comprised six questions in conversational format: (1) Describe ALL the symptoms you associate with your disease; (2) What past or present activities have been/are impacted by your disease? Any activities you can no longer do; (3) Which enjoyable/favorite hobbies or activities have been affected by your ataxia; (4) Can you think of anything in your life that has NOT been affected by your disease; (5) Do you feel your thinking/emotions have changed since you developed ataxia; (6) What symptoms/affected-activities do you feel your physicians may not routinely ask about or do not know about? Informed by expert clinician input (JDS) and literature review, the patient-reported item pool was reviewed and categorized into unique domains and subdomains based on symptoms and activities of daily living. This was used to develop a preliminary form of the PROM-Ataxia. Responses to severity or frequency questions were scored on a Likert scale 0 – 4.

Second, in the cognitive debrief validation phase we asked ataxia focus groups to assess content validity, readability, and comprehension of the questions. Focus groups were stratified (Klockgether et al., 1998): Stage 0 – normal gait, 1 – impaired gait, independent; 2 – walking aid; 3 – wheelchair. Patients completed the preliminary PROM-Ataxia individually, then discussed responses in a group setting. Ease of comprehension of each question, and relevance / importance to ataxia were scored using a Likert scale, 0 – unimportant to 4 – very comprehensible/relevant/important. We applied validation metrics for responsiveness (to severity levels) using regression coefficients (r), internal consistency of items within domains (Cronbach's alpha), and data acceptability and reliability. We amended questions in the final PROM-Ataxia incorporating feedback from these focus groups, informed by expert opinion.

Results:

147 participants (99 female) were recruited (NAF annual meeting 2018; MGH Ataxia Unit). Stage 0 – n=10, stage 1 – 75, stage 2 – 55, stage 3 – 7. 3,855 items and 49 domains were reported for the 6 questions: **1** – 1,025 items; 14 domains. **2** – 1,152; 5. **3** – 663; 5. **4** – 343; 4. **5** – 411; 12. **6** – 261; 9. 179 questions were derived from the items reported most frequently. After eliminating redundancy, we developed a PROM-Ataxia draft with 70 questions classed by 3 major domains and 14 subdomains. Physical domain (PHYS); subdomains of bulbar function, speech,

balance/coordination, fatigue, vision impairment, neuropathy, sleep, dizziness, muscle control and sexual ability. Activities of daily living domain (ADL); subdomains of activities of daily living and instrumental activities of daily living. Mental health domain (MEN); subdomains of emotional dysfunction and social health.

17 participants with ataxia (14 females) were enrolled in focus groups according to ataxia severity: stage 1 – n=6, stage 2 – 4, stage 3 – 7. Cognitive debrief showed the scale was readily comprehensible, all items were important and relevant to disease across all three domains: PHYS: 100% comprehensible, 93% important, 78% relevant; ADL: 99%, 88%, 85%; MEN: 100%, 94%, 78%. Responsiveness (correlation between total score and severity) was excellent across all domains: R values: PHYS: 0.67; ADL: 0.71; MEN: 0.51 (all $P<.05$). High levels of internal consistency and reliability, expressed as Cronbach's Alpha were PHYS: 0.94; ADL: 0.93; and MEN: 0.88. Data acceptability and reliability assessments showed that mean and median scores were consistent across all items, floor/ceiling effects showed that full range of severity is captured across all items, and all individual items were well correlated with the overall score.

Conclusions: We introduce the PROM-Ataxia derived from patients' experience of their cerebellar disorder. This 3-domain, 70-item questionnaire is readily interpretable by patients and has sound measurement properties. It is a valid and appropriate assessment of ataxia-related symptoms, capturing quality of life impact of cerebellar dysfunction on physical and cognitive-emotional abilities and on activities of daily living across the spectrum of disease severity. External validation of the PROM-Ataxia against established measures of the domains affected by ataxia is under way.

People's insights about the manifestations and impacts of ataxia of Charlevoix-Saguenay : a first step to develop patient-reported outcomes.

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Clinical trial readiness is a major issue in rare diseases in part related to the lack of outcome measures with documented metrological properties. Among the requirement, regulatory agencies' guidelines including the Food and Drug Administration emphasized the need to assess the treatment's benefits according to patient's perspective as a key outcome in clinical trials. Manifestations and impacts of the disease are assessed with a type of instrument called Patient-Reported Outcomes (PRO). Up to now, there is no PRO for any recessive ataxia, including Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS). It is therefore essential to develop a PRO that can be used specifically for this clientele. A first step to develop such instrument is to gain insights directly from the people who are affected by the condition about the concepts to measure. While this recessive ataxia is about to become the second most common in Europe, little is known about the manifestations and impacts of this disease on the daily life of people affected. The aim of this project is to document the manifestations and impacts of this disease according to the perception of people with ARSACS. Qualitative interviews have been conducted with 12 participants according to a maximum variation sampling strategy. People where ask to talk about the consequences of the disease according to the three dimensions of PROMIS conceptual framework: physical, mental and social. The 12 interviews has been transcribed and a thematic analysis has been performed. We used a codebook and a saturation table to enhance the trustworthiness. The results showed manifestations and impacts within the three dimensions. This allows us to determine what is really important for these people and which aspects of the disease have a significant impact in their daily living. These results will be useful to develop a PRO that can be use specifically with this population.

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

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 187

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Background

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

Objective

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

Methods

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

Results

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

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

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

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

Conclusions

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

Figure 1. FRDA functional impacts and their relevance to the 9-HPT[†]

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

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

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 183

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

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

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

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

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

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

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

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

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

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

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 175

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

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

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

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

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

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

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 172

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

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

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

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

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

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The spatial distribution of cerebellar and brainstem structural abnormalities in SCA1, 2, 3, and 6 from the ENIGMA-Ataxia consortium

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 170

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Spinocerebellar ataxias (SCA) are autosomal dominant inherited neurological diseases. The most common – SCA1, SCA2, SCA3, and SCA6 – are caused by CAG triplet-repeat expansions in the coding regions of their respective causative genes (e.g., *ATXN1*, *ATXN2*, etc). Although different proteins are implicated, each of the SCAs is characterized by progressive ataxia and loss of motor control secondary to neuropathology that preferentially targets the cerebellum and brain stem[1]. Structural magnetic resonance imaging (MRI) is sensitive to atrophy in the brainstem and cerebellum in both symptomatic and presymptomatic mutation carriers[2], and is more sensitive than the Scale for the Assessment and Rating of Ataxia (SARA) to track disease progression[3, 4]. However, much of the formative work to date has relied on inference in small or modestly-sized cohorts. The ENIGMA-Ataxia international neuroimaging working group has been formed to maximise the robustness and reliability of neuroanatomical characterisation of SCAs (and other ataxias) through retrospective multi-site harmonisation and aggregation of MRI data analyses (http://enigma.ini.usc.edu/ongoing/enigma-ataxia/).

In this study, results from harmonised processing of T1-weighted structural MRIs from 350 SCA mutation carriers (66 SCA1, 80 SCA2, 131 SCA3, 73 SCA6) and 392 non-carrier healthy comparators (CON) were combined across 13 sites. Volumetric changes in grey and white matter were quantified across the cerebellum and brain stem using voxel-based morphometry implemented using the SUIT toolbox in SPM12[5]. Between-group comparisons (each SCA vs. age- and sex-matched CON cohorts) were performed while controlling for age, sex, and intracranial volume (ICV). Voxel-level inference was undertaken with family-wise error correction of statistical thresholds ($p_{FWE}<0.05$) to account for multiple comparisons.

Significant grey matter volume reductions, relative to healthy controls, were evident across the entirety of the cerebellar cortex in all four diseases ($d>0.5$; Fig 1A), and across the cerebellar and brain stem white matter in all but SCA6 ($d>0.5$; Fig 1B). The magnitude and spatial distribution of this atrophy was qualitatively distinct across disorders. Global cerebellar atrophy followed the pattern SCA2 ($d=1.60$) > SCA6 ($d=1.42$) > SCA1 ($d=1.25$) > SCA3 ($d=1.03$). Atrophy was distributed relatively uniformly across the cerebellar cortex in SCA2, but weighted towards the anterior lobe in SCA1 and SCA3, and predominant in the anterior lobe in SCA6.

This large, multi-site study of brain alterations in SCA1, 2, 3, and 6 indicates that volumetric abnormalities are apparent across the entirety of the cerebellum, and - with the exception of SCA6 - the brainstem. The relative spatial distribution of atrophy, however, is disease specific between these conditions. These findings indicate that different cerebellar subunits are likely to be most sensitive to disease expression and progression across different disorders, and that the multivariate pattern of atrophy across the cerebellum may provide a useful clinical metric. These indications provide promising avenues for ongoing analyses.

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

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 169

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

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

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

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

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Blood biomarker quantification in SCA3 samples using the Neurology 4-PLEX A kit and the Simoa technology.

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 160

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Background and Objective:

Development of novel treatments for spinocerebellar ataxia type-3/Machado-Joseph disease (SCA3/MJD) will require the characterization of accessible and reliable biomarkers that could be used as outcome measures in clinical trials. Tau, neurofilament light-chain (NfL), glial fibrillary acidic protein (GFAP) and ubiquitin carboxyl-terminal hydrolase like-1 (UCHL-1) have demonstrated a role as blood biomarkers in a myriad of neurological disorders[1-8]. These four analytes can be simultaneously measured using the Neurology 4-Plex A kit in the Simoa platform (an ultrasensitive digital ELISA).

We present the results of the plasma biomarker quantification in a cohort of SCA3 patients using a Simoa assay.

Methods:

98 SCA3 patients and 30 controls were recruited according to the ESMI protocol. Demographic and clinical variables, SARA, INAS, ADL and functional scores (SCAFI, CCFS) were recorded. Plasma samples were analyzed using the Neurology 4-Plex A kit® (Quanterix)[9]. Data analysis was performed with Stata v.15.

Results:

When compared to controls, SCA3 patients showed higher levels of plasma NfL (4.03 log pg/mL vs 2.59 log pg/mL, $p<0.001$) and GFAP (4.92 log pg/mL vs 4.44 log pg/mL, $p<0.002$). Tau and UCHL-1 did not show statistically significant differences.

Multiple linear regression models were fitted to study the relationship between plasma NfL and GFAP levels with SARA, ADL and SCAFI scores, adjusting by age, gender and disease duration. SARA was a predictor for plasma concentrations of NfL (ß= 2.62, 95% CI: 0.49-4.75) and GFAP (ß=4.57, 95% CI: 1.33-7.81) (Figure 1). SCAFI showed a negative association with both NfL (ß=-11.20, 95% CI: -17.51, -4.90) and GFAP (ß=-15.74, 95% CI: -25.21, -6.27) (Figure 2). ADL showed a positive association with plasma GFAP concentrations (ß=3.30, 95% CI: 0.92-5.68).

Discussion and Conclusion:

Plasma NfL and GFAP might be useful as biomarkers of neurodegeneration in SCA3 patients, since they have shown significant relationships with disease progression scores. The analysis of longitudinal data will allow us to validate these results and clarify the role of the four analytes as potential biofluid markers.

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How to diagnose ataxia

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 158

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

Cerebellar atrophy in asymptomatic SCA1 and SCA2 mutation carriers: a clinical and structural MRI longitudinal study

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 153

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Spinocerebellar ataxias type 1 and 2 (SCA1, SCA2) are rare inherited adult-onset neurodegenerative diseases caused by expanded CAG repeats in *Ataxin1* and *Ataxin2*genes. Individuals who inherited the mutation usually remain free of symptoms for a few decades of life (preclinical stage), and then ataxic symptoms become recognizable. The extensive characterization of pathophysiological changes in the preclinical stage may allow early and more effective therapeutic interventions.

We enrolled 73 subjects belonging to SCA1 or SCA2 families: 25 symptomatic patients, 27 gene-positive preclinical subjects (preSCA), and 21 gene-negative healthy family members. We obtained ataxia clinical scores, neurocognitive data, and 3T-MRI structural measures of cerebellar lobules, subcortical nuclei and brainstem volumes, and cerebral/cerebellar cortical thickness. One-year follow-up evaluations were performed in 57 subjects.

In both SCA1 and SCA2 diseases significant trends for volume reduction, compared to controls, were identified already in preclinical phase in total cerebellar volume, cerebellar cortical thickness and in brainstem structures. Decrease cerebellar volume could be detected 8.3 years before onset in preSCA1, and 10.3 years before onset in preSCA2. Analyses of cerebellar lobules identified a different pattern of volume loss in the SCA1 and SCA2 diseases. In SCA1 atrophy was prominent in a cluster of cerebellar lobules of the posterior lobe (ρ= -0.48; p=0.001). In SCA2 disease, cerebellar atrophy appeared to be more widespread, and predominantly affecting a cluster of lobules of the anterior lobe (ρ=-0.68; p<0.001). At cerebral cortical level, a significant trend of thickness reduction in regions comprising mainly frontal and parietal regions was identified only for SCA2 disease. Follow-up confirmed the findings observed at baseline, and additionally showed volume decrease in midbrain and thalamus regions. In preclinical subjects we found significant volume reduction over time in total cerebellum volume, pons, and midbrain, despite no changes in ataxia clinical score, except for a preSCA1subject who converted to the symptomatic phase. Symbol Digit Modality Test revealed subtle cognitive deficits in symptomatic SCA1/SCA2 patients and in preSCA1.

Our study showed that cerebellar and brainstem MRI measures demonstrate high sensitivity to identify changes in a specific time-window of the pre-clinical phase of SCA. The quantification of longitudinal decline could represent a reliable biomarker of disease progression in the earliest phases of the diseases and a possible outcome measure to determine the responsiveness to therapy in trials of disease-modifying interventions.

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

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

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

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

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

Conclusion.

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

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

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

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

Dr. Gilles Naeije [1], Dr. Vincent Wens [2], Dr. Nicolas Coquelet [2], Dr. Martin Sjøgård [2], Prof. Serge Goldman [2], Prof. Massimo Pandolfo [3], Prof. Xavier De Tiège [2]

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

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

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

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

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

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

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

Quantifying the Cerebellar Cognitive Affective Syndrome in Friedreich Ataxia

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

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Introduction

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

Methods

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

Results

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

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

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

Conclusion

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

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Vestibulo-Ocular Reflex impairment in Machado-Joseph disease: A possible biomarker of the disease

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 133

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Background: Machado-Joseph disease (MJD or Spinocerebellar ataxia type 3 -SCA-3) is the most common dominant cerebellar ataxia and relatively prevalent among the Yemenite Jewish subpopulation living in Israel. Quantitative measures that would identify pre-symptomatic gene carriers at the threshold of clinical diagnosis would be extremely valuable in early diagnosis, tracking disease progression, and assessing the efficacy of new treatments. This is a crucial subject of investigation not only in MJD but also in other degenerative diseases of the nervous system.

In a pioneering study we reported vestibulo-ocular reflex (VOR) impairment that was easily detected by clinical examination in a small group of MJD Yemenite Jewish patients but not found in other types of dominant cerebellar ataxias. In further studies we confirmed that VOR deficit is part of the MJD phenotype. Nowadays, the gain of the VOR (the ratio of the eye movement response to head rotation) can be easily and accurately quantified using the video Head Impulse Test (vHIT) where fast cameras are recording the eye movement response to an abrupt head rotation. The aim of this study is to evaluate the possibility of using VOR gain as a neurophysiological biomarker of MJD.

Methods: Seventeen MJD patients and three pre-symptomatic subjects underwent a detailed clinical and laboratory neuro-otological evaluation including the Scale for the Assessment and Rating of Ataxia (SARA). Horizontal and vertical VOR gains were measured with a vHIT system.

Results: All MJD patients had significant angular VOR gain decrease (about 50% of normal values) in both horizontal and vertical planes. Ataxia severity evaluated by the SARA score was significantly correlated with the degree of VOR impairment ($r=-0.78$; $p<0.01$). All three pre-symptomatic subjects showed mild VOR gain impairment.

Conclusion: Angular VOR impairment in horizontal and vertical planes seems to be a distinctive feature of MJD. We suggest that quantitative VOR gain measured by the vHIt system could be a neurophysiologic biomarker for detecting the appearance and progression of neurodegeneration in MJD.

Ectopic Burden via Holter Monitors in Friedreich Ataxia

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

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Introduction

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

Methods

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

Results

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

Conclusions

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

Longitudinal gait and balance outcome measurement in individuals with Friedreich ataxia

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

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

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

The Friedreich's Ataxia Integrated Clinical Database

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Ms. Jane Larkindale [1], ***Ms. Jennifer Farmer*** [2], ***Ms. Diane Corey*** [1], ***Mr. Richard Liwski*** [1]

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

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

The FA-ICD contains anonymized patient-level data from the FA-COMS study, and from placebo arm data from four clinical trials (more data is being added as it becomes available). The data is in CDISC format suitable for submission to regulators. The data in the database is available to qualified researchers for free, and has been accessed 8 times in its first three months (anonymized patient-level data may be downloaded in excel or csv format and used for pre-specified research questions). Sharing of the data is directed by a steering committee consisting of the principle investigator of the FA-COMS study, a statistician with a great deal of experience in FA, an industry representative, representatives of two families with FA, as well as representatives from FARA and C-Path. This steering committee also convenes to discuss analysis that may be performed by the collaborators and shared with the community.

Increasing the characterization of FA and developing clear quantitative models of patient-level disease trajectories would allow us to better understand how disease progresses as measured by defined outcome measures and/or biomarkers in both natural history studies and in clinical trials. This in turn would aid in understanding the factors that affect the variance in progression rates and placebo affects in trial populations, and help inform development of clinical trial protocols that could more efficiently determine if a new therapeutic was effective or not. This would accelerate clinical development, make it less expensive, and encourage more companies to develop drugs for FA, in addition to many other uses of the database. The database may be accessed through http://curefa.org/researchresourcesunder FA Integrated Clinical Database.

The European Reference Network -Rare Neurological Diseases - What is in it for ataxia?

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 112

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The European Reference Network (ERN) on Rare Neurological Diseases (RND) aims to address the unmet needs of more than 500 000 people living with RNDs in Europe. Due to significant phenotype and genotype heterogeneity of RND patients, 50-60 % of those affected with rare disease are still undiagnosed and the care people receive in Europe is varied. The European Commission (EC) feels that European reference networks will help to address the needs of this population.

The ERN-RND group covers the following *6 neurological disease groups:*

-**Ataxia***& Hereditary spastic paraplegia (HSP)*

- Dystonia & Paroxysmal disorders (non epileptic ones) / Neuro-degeneration with brain iron accumulation (NIBA)
- Huntington's disease & choreas
- Rare and atypical Parkinson's including Multiple system atrophy (MSA)
- Leukodystrophies/neuro-metabolic movement disorders
- Fronto-temporal dementia

ERN-RND comprises the following working groups:

1) Diagnostic pathways
2) Care co-ordination
3) Training, education and capacity building for young neurologist in Europe
4) Information sharing and disease resources
5) Guidelines, pathways and best practice
6) Registries and research

In practice, ERN-RND cooperates with existing networks, patient advocacy groups and professional societies to improve care of RND patients in the European Commission. The ERN- RND was launched in March 2017 in Villinus, Lithuania, and has thus just started its third year of activities. It has regular virtual and face to face meetings of its daily management committee, the working groups and the disease expert groups.

In the **ataxia area,** the ERN-RND group have the following listed among their achievements for year 2:

- agreement on disease scales for ataxia and HSP
- publication of diagnostic flow charts
- endorsed guidelines
- set up a clinical management system so clinical cases can be discussed virtually in a protected environment.
- contribution to the Value of Treatment case study Ataxia, initiated by the European Brain Council.
- collecting data on care needs for Ataxia in Europe
- providing training for young fellows.

It is hoped that this will have a direct impact and improve the overall percentage of RND patients with a final diagnosis and improve their care as they struggle to cope with the everyday reality of a significant, usually progressive neurological condition.

Extensive Cerebellar and Thalamic Degeneration in Spinocerebellar Ataxia Type 10

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 110

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Spinocerebellar ataxia type 10 (SCA10) is a hereditary neurodegenerative disorder caused by repeat expansions in the *ATXN10* gene. In the same way than other spinocerebellar ataxias, SCA10 is characterized by slowly progressive cerebellar dysfunction such as limb and gait ataxia, dysarthria and ocular disturbances. The clinical diagnosis of SCA10 is mainly driven by the combination of ataxia and epileptic seizures, most often generalized motor seizures and complex partial seizures. The presence of seizures, however, is not generalizable to all SCA10 patients. Neuropathological and neuroimaging studies on the neurodegeneration pattern in SCA10 are scarce and no systematic imaging studies have been conducted to investigate the extent of SCA10-related neurodegeneration. Our aim was to characterize the gray and white matter degeneration patterns in SCA10 patients and the association with clinical features.

In this study, we enrolled 18 patients with a molecular diagnosis of SCA10 and 18 healthy individuals matched for age and sex. All participants underwent brain MRI including high-resolution anatomical and diffusion images. Whole-brain Tract-Based Spatial Statistics (TBSS) and Voxel-Based Morphometry (VBM) were performed to identify white and grey matter degeneration respectively. A second analysis in the cerebellum identified the unbiased pattern of degeneration. Motor impairment was assessed using the SARA Scale and the clinical score was correlated with the volume of grey matter and fractional anisotropy.

TBSS analysis in the patient group revealed white matter abnormalities exclusively in the cerebellum. VBM analysis showed extensive grey matter degeneration in the cerebellum, brainstem, thalamus, and putamen. Significant associations between cerebellar degeneration and SARA scores were found. Additionally, degeneration in thalamic GM and lobule VI WM were significantly associated with the presence of seizures.

The results show that besides cerebellum and brainstem, brain degeneration in SCA10 includes predominantly the putamen and thalamus; involvement of the latter is strongly associated with seizures. Analysis of the unbiased degeneration pattern in the cerebellum suggests lobules VIIIb, IX, and X as the primary cerebellar targets of the disease, which expands to the anterior lobe in later stages.

Figure 1. Parametric maps comparing SCA10 vs healthy controls for significant fractional anisotropy abnormalities (A) and its correlation with clinical scores (B, C). Significant grey matter reductions (D, E) and the degeneration signature (F).

Assessment of saccades in patients with early onset ataxia: clinical correlates

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 99

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Background: Early onset ataxias (EOAs) include both progressive (PAs) and chronic non-progressive (CAs) forms. Although cerebellar dysfunction is critical for motor disturbances of EOAs, the pathogenic mechanisms differ between PAs and CAs. Ocular movements are a paradigm of motor behavior, whose objective analysis might reflect dynamics of cerebellar functioning. Here we provided quantitative assessment of saccades in both PAs and CAs in order to evaluate the pattern of ocular motility and identify novel biomarkers for EOAs.

Methods: 10 patients with CAs, 10 with PA (Friedreich's Ataxia) and 10 sex/age-matched healthy controls have been enrolled in this cross-sectional study. Subjects underwent standardized clinical evaluation with Scale for the assessment and rating of ataxia (SARA), Time 25-Foot Walk (T25FW) for velocity and 9-Hole Peg Test (9-HPT) for dominant hand dexterity. Saccades were assessed by infrared oculography (EyeSeeCam) during different plane-restricted tasks (horizontal and vertical gaze). The following saccadic parameters were analyzed: latency, amplitude, duration, peak velocity, and peak velocity/amplitude ratio (so called "main sequence") that is considered a specific marker of cerebellar functioning. Data were compared among the groups and correlated with clinical scores.

Results: PA and CA groups were homogeneous in demographic and clinical features (SARA, T25FW scores), whereas the pattern of saccadic impairment differed. Namely, CA patients compared to controls had increased latency and reduced amplitude and peak velocity. PA patients had only higher latency. The "main sequence" in vertical gaze was lower in CA patients than PA patients and controls, while in horizontal gaze was greater in PA patients than CA patients and controls. In CA group, amplitude of vertical saccades was inversely related to SARA; in PA group amplitude of horizontal saccades tended to be directly correlated with SARA.

Discussion: This study explored quantitative saccadic parameters in patients with EOA demonstrating distinct patterns of impairment in PAs and CAs. In particular, we found a prominent impairment of vertical saccades in CAs, proportionate to global ataxia, and a preferential disruption of horizontal saccades in PAs. Such a segregation may suggest the different involvement of underlying neural circuits, consistent with typical neuropathology of PAs and CAs.

Conclusions: Differences in saccadic abnormalities of CAs and PAs patients probably reflect distinct patterns of impairment in oculomotor network, which provide some insights on the pathophysiology of the conditions and the potential use of saccadic parameters as clinical endpoints. Actually, the objective measurement of saccades in different tasks by non-invasive oculography might offers a number of indexes, coherent with clinical severity and different etiology of the diseases, which could represent novel biomarkers for EOAs.

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

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

Ms. Hannah Reece [1], Ms. Geneieve Tai [2], Ms. Hannah Carter [3], Dr. Louise Corben [2], Prof. Martin Delatycki [2], Dr. Adam Vogel [4]

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

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

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

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

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

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

The Friedreich Ataxia Impact Scale (FAIS) is a quality of life tool that was developed to address specific issues related to disease impact in people with Friedreich ataxia. The FAIS comprises 126 items and three alternative shorter versions of this tool have been proposed[1]. The FAIS-OBS was developed for clinical-based research or observational studies, the FAIS-MORE was designed for studies examining people with a more severe disease, and the FAIS-LESS for studies comprising individuals with a milder disease. The FAIS was previously studied over a period of one and two years and demonstrated limited responsiveness to change[2]. This finding could be attributed to the FAIS losing its sensitivity when administered to a heterogeneous population of varying disease severity.

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

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

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

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Health Related Quality of Life in Friedreich Ataxia

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

Ms. Emily Xiong [1], ***Ms. Abigail Lynch*** [1], ***Ms. Jennifer Farmer*** [2], ***Dr. Christian Rummey*** [3], ***Dr. David Lynch*** [4]

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

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

Results: Subjects were on average young adults (mean age=32.4 +13.6 years), with disease onset as teenagers (16.2 + 10.2 years), and disease duration of 16.1 years. In cross sectional analysis of the SF36, the subscale with the lowest HRQOL score was the physical function scale (27.6 +26.6). The emotional well-being score was the highest among SF36 subscores (67. 5 +17.8). We also examined correlations of SF36 scores with disease features. The physical function scale correlated with age of onset (P<0.0001), duration (P<0.0001) and age (P<0.0001). The physical role limitations-physical scale correlated with age of onset and duration; the role limitations/emotional scale correlated with disease duration (P=0.0249) and age (P=0.0151); the pain scale with duration (P= 0.002) and age (P=0.0382); and the change in health correlated only with age of onset (P=0.0046). In assessment of symptom specific scales, bladder control scores (BLCS) correlated with duration and age, while impact of visual impairment scores (IVIS) correlated with duration. In linear regression models, BLCS, Pain Effect Score (PES), and IVIS scores were predicted by age and GAA repeat length; Modified Impact Fatigue Scale (MFIS) scores were predicted only by GAA repeat length.

We also examined the change in SF-36 and symptom specific scores over time. Physical function and role limitation scores (both emotional and physical) declined over time. Although initial scores for these subscores were slightly lower in subjects with early onset, the rate of change was similar in subjects with different ages on onset. Essentially no change was seen over time in any of the other subscores. Symptom specific scales also worsened over time; this was most notable for the IVIS and BLCS, with less consistent change in the MFIS and PES. For the IVIS, the decline was far more prominent in individuals with onset at age 14 or below. For other scores progression was similar in subjects stratified by age of onset.

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

Personalized Digital Biomarkers to Forecast and Longitudinally Track Ataxic Gait Patterns Across Different Neurological Disorders

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 46

Prof. Elizabeth Torres [1]

1. *Rutgers University*

Several neurological disorders manifest ataxic symptoms that appear at different stages of the human lifespan. Some symptoms manifest rather early in disorders like autism of idiopathic origins and of known etiology, and persist throughout life, worsening as the person ages. Others appear midlife, as in Fragile X related disorders such as those present in pre-mutation carriers, and yet others appear later in advanced age, as in many patients with Parkinson's disease. In all cases, it is desirable to measure the problem with high precision and in personalized manner to detect and forecast the symptoms before they appear, and to track them as the disorder progresses. Further, in all cases it is desirable to have outcome measures capable of tracking treatments' effectiveness longitudinally, e.g. during clinical trials.

Here we introduce a new data type coined micro-movement spikes paired with a personalized analytical platform that permits the assessment of fluctuations in biorhythmic activities self-generated by the nervous systems. We apply the methods to waveforms harnessed across multiple functional layers of neuromotor control. These occur along an orderly taxonomy of control spanning from voluntary (deliberate) movements to spontaneous motions consequential of goal-directed behavior, to automatic, involuntary and reflexive patterns. Using new methods, we co-register different data streams from multiple sensor types and can track change along stochastic trajectories reflecting the shifts in the probability landscape of various movement parameters of relevance.

We present examples to illustrate our models and data type using the results from a clinical trial of IGF-1 in 16 children with SHANK3 deletion syndrome whereby we study the evolution of gait patterns across the drug trial. Further, we compare these gait patterns with age- and sex-matched controls and with 15 children with idiopathic autism who did not participate in the trial. We also illustrate the methods in 20 participants with FX-related syndrome and in 20 patients with PD to build a map in probability space whereby we determine patients' locations and distances to other patients, as self-emerging clusters manifest in a heuristic-free model. Because these digital data are informed by traditional clinical inventories like the UPDRS, we provide new standardized statistical scales amenable to reproduce results across labs and patient populations.

This work offers a new way to track change over time using appropriate statistical methods, metric spaces and protocols amenable for use in personalized medicine within the laboratory settings and / or at home and the clinic.

Predictors of Loss of Ambulation in Friedreich's Ataxia

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

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

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

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

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

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

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

Psychometric Properties of the Friedreich Ataxia Rating Scale

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

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

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

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

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

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

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

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

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

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

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

Objectives: To utilize interviews with individuals with FA and caregivers of young people with FA to identify the symptoms that generate the greatest disease burden in the FA population.
Methods: We interviewed individuals with FA aged 11 years and older and caregivers of children aged 0 to 10 years with FA. Using open-ended questions, participants with FA identified the symptoms and issues that have the greatest impact on their lives. Caregivers were asked to identify the symptoms and issues that they observe to have the greatest impact on their children's lives. Interviews were recorded, transcribed, and will be analyzed using a qualitative framework technique, triangulation, and a three investigator consensus approach.
Results: We interviewed 23 individuals with FA and 7 caregivers of young people with FA. Eight of the individuals with FA were minors. Participants represented 19 states across the United States. Additional interviews are currently underway and will be conducted until concept saturation has been reached. The collective data from all patient and caregiver interviews, including quote and symptom frequency, will be reported at the time of the meeting and will include insight into disease burden from caregivers and individuals with FA of different ages and demographics.
Conclusions: There are many symptoms and symptomatic themes that impact the lives of individuals that live with FA. Affected individuals and caregivers are uniquely suited to provide granular input into the symptomatic burden experienced by individuals with FA. Data regarding what symptoms generate the greatest burden is useful as researchers plan studies in FA, clinicians take care of individuals with FA, and as relevant disease-specific outcome measures are developed for this population.
Acknowledgements: Funding for this project was provided by the Friedreich's Ataxia Research Alliance (FARA) and the project was developed in collaboration with the FARA PRO working group.

Automated Spinal Cord Segmentation in Inherited Ataxic Diseases

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 71

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Introduction: Spinal cord (SC) atrophy is a hallmark of several inherited ataxia diseases (IAD). However, SC imaging in research and clinics is still largely underutilized, because it is difficult to acquire good quality data due to the numerous artifacts and the small cross-sectional size of the SC, but recent MRI advances now enable reliable quantitative SC analyses. The Spinal Cord Toolbox (SCT) is a validated software package specifically designed to process SC multimodal MRI data. This new technique provides reliable measures of cross-sectional areas for both WM and GM tissues, enabling researchers to better understand the pathogenesis of IAD.

Methods: We enrolled 122 patients (45 cFRDA, 41 SCA3, 29 SCA1 and 7 SPG7) and 148 age-gender matched healthy controls. All subjects underwent magnetic resonance imaging in a 3T device, three-dimensional high resolution T1-weighted (cFRDA, SCA3 and SCA1) centered in the brain but including the upper cord, and 2D T2* images (SPG7) covering C2 to C4 vertebral levels were used to assess the total cross-sectional area (CSA) and gray matter (GM) CSA respectively. We used SCT to automatically quantify the total and GM CSAs for C2 and C3 vertebral levels. We assessed only these vertebral levels because we used cerebral T1-weighted images to assess the cervical SC and lower levels, in these images, were not acquired or presented low signal-noise-to-ratio. All analyses were corrected for multiple comparisons.

Results: SCT showed great performance for cord segmentation and vertebral labeling and manual correction was necessary in less than 5% of all images (Figure 1). Group comparison for T1-weighted images showed that the total CSA was reduced in all groups when compared to their respective control groups. Regarding the inherited spinocerebellar ataxias, we could reproduce our previous published findings with comparable measurements (FRDA: SCT=42.8±5.7 and SpineSeg=37.1±5.2; SCA3: SCT=44.3±6.1 and SpineSeg=49.5±7.3; SCA1: SCT=48.3±5.8 and SpineSeg=47.3±5.9). For SPG7, which were assessed by T2* images, we found the total CSA reduced in comparison to their matched healthy controls. However, there is no study assessing the spinal cord area in such patients that allowed to compare the measurements. Regarding the GM CSA (Figure 2), we did not find any significant result when compared the patients to their matched controls. SPG7 patients only showed damage in the WM spinal cord. We found significant correlation between CSA and disease severity or duration for FRDA (FARS: R=-0.654, p<0.001; Duration: R=-0.457, p=0.011), SCA3 (SARA: R=-0.472, p=0.002; Duration: R=-0.484, p=0.001) and SCA1 (SARA: R=-0.594, p<0.001; Duration: R=-0.381, p=0.045) patients.

Conclusion: The SCT software showed similar neuroimaging findings in comparison to previous studies, bringing new insights, enabling us to assess different vertebral labels and tissues (GM and WM). SCT is free and open source, compatible with any scanner brand, using images in NIfTI format and is designed to accept a variety of sequences, modalities and contrasts. Therefore, SCT is a useful tool specifically developed to assess SC images that might help us to investigate the real role of SC as neuroimaging biomarker in many neurodegenerative disorders.

Figure 1: Example of automatic spinal cord segmentation and vertebral labeling by SCT in a T1-weighted imaging.

Figure 2: Example of automatic gray matter segmentation of the cervical spinal cord in a T2*-weighted imaging.

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

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

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

ARSACS cognitive profile: correspondence with cerebellar cognitive affective syndrome?

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 79

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The cerebellar cognitive affective syndrome (CCAS) described by Schmahmann and Sherman (1998) is characterized by impairments in the cognitive and affective domains, including working memory and visuospatial organization skills.

This syndrome was identified for diseases involving cerebellar dysfunction. Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a degenerative spinocerebellar disease. A pilot study on central nervous system involvement in this population found indicators of cognitive deficits such as information processing speed, sustained attention, language function, and visual logical reasoning, which could resemble what is found in the CCAS. Clinicians also noted difficulties worsening over time for individuals with ARSACS related to increasing difficulties to learn new tasks and understanding others point of view.

Objective: To explore the presence of the CCAS in ARSACS by documenting the main components of this syndrome including visual working memory, verbal learning, visuospatial and visuoconstructive skills, reaction time and socio-cognitive deficits.

Methods: This study investigated 30 adults with ARSACS (c.8844delT mutation; aged between 20-59 years old). They were assessed using preselected neuropsychological tests, including social cognition with comparison with normative data when available. Results: The results suggest a diffuse pattern of visuospatial and visuoconstructive function impairments in ARSCS. Indeed, great inter-individual differences were found between participants. Performances tended to be worse with older participants. Also, 54 % of our sample got lower results as compared to expected performances in facial recognition assessment. For visual working memory, only 51 % had results in the normal range. Evaluation of verbal memory showed that 80 % are in the deficit area the total number of words rehearsed in the learning phase, and 75 % got results classified into this same area for immediate and long-term verbal memory. The mean reaction times were abnormally slow for 72 % of the participants. Considering the theory of mind, 46 % of the participants showed a deficit. In fact, most of them were able to have a good comprehension of the awkward situation and to attribute the exact emotion to the victims. However, half of them had a result in the deficit range when they had to elaborate on intention comprehension.

Conclusion: Deficits in processing speed, working memory, and visuospatial and visuoconstructive skills described in CCAS were observable among our ARSAC participants. However, several results below expected performance were found more often for participants over 40 years old. Wide inter-individual differences were found with regard to performances on psychometric tests. This could be explained by other factors (i.e.: variability of the physiological and physical evolution of ARSACS). New studies, including MRI scan and longitudinal data on cognitive evaluations will be required to understand natural evolution of the disease in ARSACS.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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Clinical trial readiness in spinocerebellar ataxias

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

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Spinocerebellar ataxias (SCAs) are a group of rare autosomal dominant diseases presented with ataxia. European investigators of the Ataxia Study Group (ASG) have conducted longitudinal natural history studies including EUROSCA and RISCA studies on SCA1, SCA2, SCA3 and SCA6. The US Clinical Research Consortium for Spinocerebellar Ataxias (CRC-SCA) also conducted similar studies in the corresponding US SCA cohort. Under the European SCA3/MJD Initiative (ESMI) further natural history data of a greater number of SCA3 subjects have been collected while the READISCA study has been initiated focusing on early-stage patients and premanifest carriers of SCA1 and SCA3 mutations in the US and Europe under the NIH funding. Although no disease modifying treatments for SCAs are currently approved by the FDA or the EMA, several therapeutic approaches are anticipated to come to clinical trials in the near future. The READISCA will determine the progression rate of ataxia and associated manifestations using validated clinical outcome assessment (COA) measures, such as the Scale for Assessment and Rating of Ataxia (SARA), Composite Cerebellar Functional Severity (CCFS), and Inventoryof Non-Ataxia Signs (INAS), and evaluate disease progression biomarkers based on brain magnetic resonance imaging/spectroscopy and biofluid analyses for their usefulness in future clinical trials while establishing large cohorts of early stage subjects with SCA1 and SCA3. The READISCA is also collecting biosamples (DNA, plasma, CSF and peripheral blood monocytes) from these subjects. The COA instrument to evaluate cerebellar cognitive affective syndrome (CCAS) and wearable sensors for quantitative and continuous ataxia assessment are tested. These data will be used in computer simulations of emerging clinical trial designs. A parallel longitudinal study of subjects with SCA1, SCA2, SCA3, SCA6, SCA8 and SCA10 with all stages of the disease (CRC-SCA Natural History Study) is collecting COA data and biofluids with funding from the National Ataxia Foundation. This year, the SCA Global was established with participation of SCA investigators worldwide, and the 1stPan-American Conference on Hereditary Ataxia was held in Havana. While international studies are challenging, it is critically important for clinical trials of rare diseases such as SCAs.

Changes in speech timing and swallowing function are detectable at the pre-ataxic stage of Spinocerebellar Ataxia Type 2

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 14

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Background: Speech and swallowing deficits are common in spinocerebellar ataxias (SCAs) yet the nature and severity of SCA genotype-specific deficits, in particular their evolution at the pre-ataxic to the early-manifest stages are not well characterized. Data are lacking almost completely for SCA2. Comprehensive objective and qualitative assessment of dysarthria and dysphagia in SCA2 specifically of the earliest disease phases will pave the way not only for timely targeted neurorehabilitative interventions, but also for outcome measures in future treatment trials and stratification of the pre-ataxia conversion phase, which offers a unique opportunity for preventive therapies.

Methods: Thirty-six individuals (16 pre-ataxic SCA2, 14 ataxic SCA2 at early stage and 16 healthy control) were recruited in Holguin, Cuba. All participants underwent a comprehensive battery of assessments including objective acoustic analysis, subjective clinician derived ratings of speech function and swallowing, and quality of life related exams of swallowing.

Results: Speech deficits manifest from reduced diadochokinetic rate, increased diadochokinetic period at the pre-ataxic stage, with increased changes at the early-ataxic stage. Speech rate was slower in early ataxic SCA2 compared with pre-ataxic SCA2 and healthy controls. Reduced speech agility and speech rate were both associated with disease severity, suggesting that aspects of speech timing change with disease progression. Perturbation of DKK period and slower speech rate also correlated with time to ataxia onset, confirming speech deficits occurred prior to ataxia onset and increased in severity as the disease progresses. Whilst dysphagia was observed in both pre-ataxic and ataxic SCA2, it was not associated with swallowing-related quality of life, disease severity or time to ataxia onset.

Conclusions: Speech and swallowing deficits appear sensitive to disease progression in early-stage SCA2, with syllabic rate a viable marker even in pre-ataxic stages. Findings provide insight into the underlying mechanisms of disease progression in early-stage SCA2 and signal an opportunity for stratifying early-stage SCA2 patients and identifying salient markers of disease onset as well as outcome measures in future early-stage treatment intervention studies.

Use of Antidepressants in the FA-COMs Natural History Study

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

Dr. Christian Rummey [1], ***Ms. Jennifer Farmer*** [2], ***Dr. Louise Corben*** [3], ***Dr. David Lynch*** [4]

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

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

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

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

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

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

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

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Figure 1 - Use of Antidepressant Medication by Disease Duration; bar height depicts % of patients, absolute numbers are given on top of the bars

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

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

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Background:

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

Methods:

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

Results:

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

within a corticospinal tract region-of-interest.

Conclusions:

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

DRPLA - Our Journey to Find a Treatment

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 308

Ms. Andrea Compton [1], ***Mr. Paul Compton*** [1], ***Ms. Lyndel Moore*** [1], ***Mr. Mark Adamson*** [1], ***Ms. Robyn Adamson*** [1], ***Ms. Sonia Moore*** [1]

1. *CureDRPLA*

My son was diagnosed with Juvenile onset dentatorubral-pallidoluysian atrophy or DRPLA in August 2018. This poster will inform about the disease and the steps undertaken to create a DRPLA community and find a treatment. DRPLA is an ultra-rare autosomal dominantly inherited disorder caused by a CAG trinucleotide repeat expansion (>48 tandem copies) in the Atrophin-1 ("ATN1") gene. DRPLA occurs more commonly in Japan where the prevalence is estimated at 0.48:100000. Life expectancy is 8-16 years from symptom onset.
We undertook the following steps to better understand DRPLA and the pathways for a cure:

1. Found key scientific experts, learned about any past or present DRPLA research, contacted authors of DRPLA research and rare genetic disease information/support organizations. Members from the CureDRPLA network attended, spoke at and/or exhibited at the following 2019 conferences: Global Genes RARE Drug Development Symposium; Global Genes Patient Advocacy; the International Movement Disorders Society; the Manchester Rare Disease Showcase and the AtaxiaUK annual conference.
2. Using RareConnect, which translates into 13 languages including Japanese, we found approximately 60 other people with DRPLA who could potentially be involved in clinical trials. We continue to broaden this network.
3. Created a DRPLA logo, website and foundation for funding disease research.
4. Liaised with biotech companies to assess interest in developing a drug for treatment of DRPLA. Through our work in this area, we established 3 separate consortiums. These consortiums include uniQure, Weill Cornell Medical College, Dr Shoji Tsuji, Ionis Pharmaceuticals, Giovanni Stevanin (Hôpital Pitié-Salpêtrière, Paris) and Manolis Fanto (Department of Basic and Clinical Neuroscience Institute of Psychiatry, Psychology & Neuroscience, King's College London) and others.
5. Identified opportunities for future cultivation of the DRPLA community.
6. Created RFPs for a Natural History Study and DRPLA Biomarker study to be vetted and administered by AtaxiaUK.
7. Created a patient contact registry.

Staying Strong Toolbox for Aboriginal families with MJD in the Top End of Australia

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 164

Ms. Jen Carr [1]

1. *James Cook University*

Aboriginal people from Groote Eylandt and Ngukurr have developed the *Staying Strong Toolbox* for individuals and families living with Machado Joseph Disease (MJD) to keep them walking and moving around for as long as possible. While there is currently no cure for MJD, physical activity has been found to enhance mobility for individuals with degenerative ataxias. However, there has been limited research on mobility specific to MJD and no research on mobility for Aboriginal families with MJD in Australia. As a result, families with MJD from Groote Eylandt invited researchers to collaborate with them to identify the best ways to keep 'walking and moving around'.
This presentation will outline the community driven process and collaborative partnerships that have led to the co-design of the Staying Strong Toolbox; a physical activity and lifestyle program for Aboriginal families with MJD. Community researchers from Groote Eylandt and Ngukurr worked together with a university researcher to design the *Staying Strong Toolbox*by:

1. Identifying 'what works best' to keep families with MJD 'walking and moving around' from the perspective of Aboriginal families with MJD and research gathered from overseas.
2. Putting the ideas together to develop the toolbox.
3. Piloting the toolbox on Groote Eylandt and in Ngukurr

The *Staying Strong Toolbox*was recently piloted and found to have a positive impact on families living with MJD on Groote Eylandt and in Ngukurr. This finding suggests that the 'Toolbox' has the potential to reduce the impact of MJD on families around the world, keeping them living a good life for longer. This story may strengthen future collaborative research in other communities, both in Australia and internationally.

Topic: Clinical Trials and Clinical Trial Design

Trunk and lower limb control for better mobility: Assessment of a rehabilitation program in Autosomal recessive spastic ataxia of Charlevoix-Saguenay

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 214

Mrs. Isabelle Lessard [1], ***Prof. Cynthia Gagnon*** [1], ***Mrs. Viviane Masterman*** [2]

1. *Faculté de médecine et des sciences de la santé, Université de Sherbrooke, Québec, Canada,* ***2.*** *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*

Introduction: Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a hereditary neurological disorder presenting with pyramidal (i.e. lower limbs spasticity), cerebellar (i.e. incoordination) and neuropathic (i.e. distal muscle weakness) impairments. Our previous results have shown that people with ARSACS have major impairments in regard to upper and lower limbs coordination, upper limbs dexterity, walking speed/endurance and balance control, ultimately leading to participation restrictions and difficulty to perform activities of daily living. No cure exists for ARSACS, at the moment we can only alleviate deficits. However, no study has been published in the scientific literature regarding rehabilitation interventions for this population. Nevertheless, there are some study conducted in degenerative ataxia other than ARSACS, which have documented positive effects of physical therapy on balance, gait and performance of daily living activities.

Methods: The objective of this pilot project was to document the effects of a rehabilitation program aiming to increase trunk and lower limb motor control on walking capacities, balance and accomplishment of daily activities in people with ARSACS. This pilot experimental study used a pre-post design. Participants were assessed before the intervention (T0), after 4 weeks (T1) and after the 8-week rehabilitation program (T2). Many outcomes measures were used, including the 10-Meter Walk Test, the Six-Minute Walk Test, the Berg Balance Scale, the Ottawa sitting scale, the Lower Extremity Motor Coordination Test and the 30-Second Chair Stand Test. Randomized sampling, stratified by age and sex, was used with the aim to recruit a total of 10 participants. The rehabilitation program was conducted by two physical therapists and two physical therapists students and consisted of 3 sessions per week for 8 weeks. At each week, one session was performed in the pool, and the two others in the training room.

Results: Statistical analyses are currently in progress and will be presented at the conference. However, some notable improvements were observed during the project, including an increase in the difficulty level of balance and trunk control exercises throughout the project for all participants. In addition, a change in the walking aid used in the home was noted for three participants. We also observed an improvement of the ability to get up from a chair and participants reported a decrease in the frequency of falls.

Speech treatment for people with hereditary ataxia – a feasibility study

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 300

Prof. Anja Lowit [1], ***Ms. Aisling Egan*** [1]

1. *Strathclyde University*

Speech treatment for people with hereditary ataxia – a feasibility study

Hereditary ataxias are frequently associated with ataxic dysarthria. The characteristics of ataxic dysarthria include imprecise articulation, distorted vowels, hypophonia, reduced speech rate, flat prosody and poor respiratory support. These changes lead to reduced speech intelligibility and a reduced effectiveness of communication which can affect quality of life. Communication breakdown has been ranked amongst the top three most upsetting symptoms of their disease by people with Friedreich's Ataxia in a recent survey by Ataxia UK.

Whilst we have significantly increased our understanding of the nature of the communication problems experienced by speakers with ataxic dysarthria, there are very few intervention studies and a recent Cochrane review concluded that "there is insufficient and low or very low quality evidence from either RCTs or observational studies to determine the effectiveness of any treatment for speech disorder in any of the hereditary ataxia syndromes." (Vogel et al. 2014, p.1).

Based on the speech symptoms prevalent across the various types of hereditary ataxia, one treatment approach that has potential to increase communication efficiency is Lee Silverman Voice Treatment (LSVT). This treatment approach focuses on increasing the level of loudness in a person's speech. The method has been shown to positively affect the wider articulatory system, such as improving breath support for speech, slowing down rate, and improving voice quality and articulation, whilst at the same being simple enough for the patient to implement in everyday communication. There are many reports , including RCTs (Baumgartner et al. 2001, Ramig et al. 2018), showing the benefits of LSVT for people with Parkinson's Disease (PD), as well as smaller studies on other disorders such as cerebral palsy (e.g. Boliek & Fox 2014), traumatic brain injury and stroke (Wenke et al. 2008, Mahler & Ramig 2012). In addition, a single case study on a patient with ataxic dysarthria as a result of a thiamine deficiency demonstrated improvement in overall speech intelligibility following a course of LSVT (Sapir et al., 2003), highlighting its potential as a treatment for speakers with hereditary ataxias. However, further evidence about its effectiveness across a larger number of participants and a wider range of underlying neuropathologies is necessary before LSVT can be advocated as an appropriate treatment for people with ataxic dysarthria.

We report on a feasibility study that aimed to investigate the effectiveness of LSVT to improve communication efficiency, and the acceptability of the approach to people with hereditary ataxia.

We recruited 20 participants with hereditary ataxia and dysarthria (17 FRDA, one SPG7, one SCA6 and one unspecified cerebellar ataxia). 19 of these concluded treatment and all assessment points. Dysarthria severity levels ranged from mild to severe. Participants were offered extended LSVT on a 2 session a week basis over 8 weeks via Skype. Assessments included 2 baseline tests, as well as immediate and 6-8 week post treatment follow-ups, also conducted via Skype. Analysis included a range of speech measures, as well as qualitative assessments of voice handicap, communication participation, and fatigue, captured by rating scales as well as interviews.

The interview data indicate that 16 of the 19 participants perceived considerable improvements to their communication following LSVT intervention, whilst three reported only minor improvements or no change. Prominent themes emerging from the interviews include the ability to produce longer utterances and speak for longer periods of time, decreased hypophonia, reduced anxiety and greater confidence. A further twelve participants also reported

improvements in intelligibility, clearer speech and/or a reduced need to repeat themselves. The formal rating scales corroborate these qualitative results to some degree with the majority of participants showing improved scores after treatment, although this did not reach statistical significance due to variable group performance. Perceptual evaluations of intelligibility and naturalness of a reading sample showed similar results, with some participants showing improved scores after treatment, but not to the extent expressed in the qualitative evaluation. Acoustic voice analysis demonstrated statistically significant performance improvements following treatment, with increases in prolonged vowel length and reduced voice perturbation (jitter and shimmer) values. These effects were maintained 8 weeks post-treatment.

Our study provides clear support for offering speech treatment such as LSVT for people with degenerative ataxia to improve their communication and psychosocial wellbeing. This finding is corroborated by a recent pilot study into intensive home-based treatment involving seven speakers with autosomal recessive spastic ataxia Charlevoix-Saguenay (ARSACS) by Vogel et al. (2019), which identified improvements in intelligibility and naturalness post-treatment.

Scale for the Assessment and Rating of Ataxia (SARA): appropriate for clinical trials of Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS)?

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 266

Mr. Dax Bourcier [1], ***Prof. Mathieu Bélanger*** [2], ***Ms. Isabelle Côté*** [1], ***Prof. Cynthia Gagnon*** [3]

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BACKGROUND: The Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS) is paediatric-onset slowly progressive neurodegenerative disease presenting with cerebellar (upper and lower limbs ataxia), pyramidal (lower limbs spasticity) and neuropathic signs (upper and lower limbs distal weakness). To date, no curative treatment is available for the 320 patients suffering from this rare disease in Eastern Canada. However, recent findings on the pathophysiology and availability of a mouse model increases the likelihood that clinical trials will be initiated in the near future. Therefore, it is of crucial importance to document outcome measures to describe and quantify the progression of ARSACS in preparation for clinical trials, as emphasized by the Food and Drugs Administration. The Scale for the Assessment and Rating of Ataxia (SARA) measures cerebellar ataxia and is commonly used by neurologists and researchers worldwide. The SARA includes eight items with a total score that can vary from no ataxia (score of 0) to most severe ataxia (score of 40). It is a generic scale that takes 4 minutes for experienced clinicians to administer, has been validated in over 13 different ataxic diseases, but has yet to be documented in ARSACS. This is a key step to pursue as ARSACS patients present with a different clinical picture than the populations in which the SARA has been validated.

OBJECTIVES: The two main objectives for this study were to 1) document the content and construct validity and 2) assess the responsiveness and clinical interpretability of the SARA over a five-year period in the ARSACS population.

METHODS:A methodological study was conducted from the secondary analysis of the largest longitudinal study in ARSACS of 100 participants over a 5-years period. The inclusion criteria were to be between 14 and 59 years old with a genetic diagnosis of ARSACS, and the exclusion criteria that there were no other known medical conditions with functional deficits. A random sampling strategy stratified by age group and sex was used. The data collection was done with outcome measures known to have excellent validity and reliability in ARSACS, and administered according to standard operating procedures at a Neuromuscular Clinic. The concepts of interest include the severity of the disease (measured with SARA, DSI-ARSACS and SPRS), motor function (measured with SFNT, LEMOCOT, 10mWT, TUG and 30CST), balance (measured with the BBS), and lastly the overall impression of change (with a scale that documents the global impression of change a participant has experienced for each symptom over the assessment period).

RESULTS: Preliminary results demonstrate that the SARA was able to detect ataxia progression beyond the standard error of measurement (SEM) over a 2-year period. Conclusive results to the following hypotheses will be available by the IARC Conference. The SARA will show good construct validity with absolute correlations (*r* coefficients) of >0.6 with the SFNT, >0.7 with the Barthel Index and 10mWT, >0.8 with the BBS, 30-CST, TUG, LEMOCOT and SPRS and >0.9 with the DSI-ARSACS, and will be able to distinguish between age group and disease stage. The SARA will show limitations in the content validity for its assessment of lower limb ataxia, but excel in its ability to detect and quantify disease progression over time beyond the SEM and the minimal important change (MIC).

CONCLUSION: The SARA is expected to be an overall valid and responsive scale to assess ataxia severity in the

ARSACS population. The involvement of pyramidal and neuropathic manifestations in lower limb ataxia SARA scores should be taken into consideration for clinical interpretation. The SARA is anticipated to able to detect change beyond the SEM and the MIC over a 5-year period making it an appropriate scale for clinical trials aiming to use cerebellar ataxia as a concept of interest.

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

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

Dr. Dmitri Volfson [1], ***Dr. Greg Hather*** [1], ***Dr. Jonathan Norton*** [1], ***Prof. Fay Horak*** [2], ***Mr. Matthew Johnson*** [3], ***Dr. Elena Izmailova*** [4], ***Ms. Jennifer Farmer*** [5], ***Dr. David lynch*** [6], ***Dr. Hao Wang*** [1]

1. *Takeda Pharmaceuticals,* ***2.*** *Department of Neurology, Oregon Health and Science University,* ***3.*** *APDM Inc,* ***4.*** *Koneksa Health,* ***5.*** *Friedrich's Ataxia Research Alliance,* ***6.*** *Children's Hospital of Philadelphia*

Background

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

Methods

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

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

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

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

Results

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

Conclusion

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

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

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

Dr. Charmaine Demanuele [1], ***Dr. Moore Arnold*** [2], ***Ms. Katherine Beaverson*** [3], ***Mr. Allen Best*** [4], ***Ms. Hannah Casey*** [5], ***Dr. Lawrence Charnas*** [3], ***Mr. Matthew Czech*** [1], ***Dr. Jeremy Edgerton*** [6], ***Dr. Mahmoud El-Gohary*** [7], ***Dr. Victoria Irzhevsky*** [8], ***Mr. Matthew Johnson*** [7], ***Dr. Isik Karahanoglu*** [1], ***Dr. Sheraz Khan*** [6], ***Dr. Robert Mather*** [1], ***Prof. Susan Perlman*** [9], ***Dr. Vesper Ramos*** [1], ***Dr. Liana Rosenthal*** [10], ***Prof. Jeremy Schmahmann*** [11], ***Ms. Joan Severson*** [4], ***Dr. Ludy Shih*** [8], ***Dr. Koene Van Dijk*** [1], ***Prof. Fay Horak*** [12], ***Prof. Christopher Gomez*** [5]

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

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

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

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

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

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

Ms. Jane Larkindale [1], ***Ms. Jennifer Farmer*** [1], ***Prof. S H Subramony*** [2], ***Dr. Louise Corben*** [3]

1. *Friedreich's Ataxia Research Alliance,* ***2.*** *University of Florida,* ***3.*** *Bruce Lefroy Centre for Genetic Health Research, Murdoch Children's Research Institute*

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

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

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

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

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

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

Mr. Frederic Heerinckx [1], ***Mr. Oliver Tucher*** [1], ***Dr. Peter Milner*** [1], ***Dr. Robert Molinari*** [1], ***Ms. Sarah Endemann*** [1], ***Dr. Mikhail Shchepinov*** [1], ***Dr. Harry Saal*** [1], ***Dr. Mark Midei*** [1]

1. *Retrotope*

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

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

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

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

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

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

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

Dr. Mary Kearney [1], ***Prof. Massimo Pandolfo*** [2]

1. *Irish College of General Practitioners,* ***2.*** *Université Libre de Bruxelles*

Friedreich ataxia is a rare, slowly progressive disorder. Researchers have not yet defined a “perfect” clinical marker or a bio-marker to assess disease progression.
Clinical assessment, using this ataxia rating scale is the current clinical marker. However the accuracy of the ataxia rating scales, is questionable when the patient is wheelchair-dependent. In view of this and the slowly progressive nature of Friedreich ataxia, large, randomized controlled trials (RCT) of at least 12 months’ duration are needed to evaluate the effects of pharmacological treatments in Friedreich ataxia.

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

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

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

Dr. Marinela Vavla [1], ***Dr. Grazia D'Angelo*** [2], ***Dr. Filippo Arrigoni*** [2], ***Dr. Nicola Toschi*** [3], ***Dr. Denis Peruzzo*** [4], ***Dr. Sandra Gandossini*** [2], ***Dr. Annamaria Russo*** [2], ***Mrs. Eleonora Diella*** [2], ***Dr. Stefania Tirelli*** [2], ***Dr. Roberto Salati*** [2], ***Dr. Paolo Scarpazza*** [2], ***Dr. Riccardo Luffarelli*** [2], ***Dr. Silvia Fortuni*** [3], ***Dr. Alessandra Rufini*** [3], ***Dr. Ivano Condò*** [3], ***Prof. Roberto Testi*** [3], ***Dr. Andrea Martinuzzi*** [2]

1. *University of Padua,* ***2.*** *IRCCS E Medea Scientific Institute,* ***3.*** *University of Tor Department of Biomedicine and Prevention, University of Rome "Tor Vergata,* ***4.*** *IRCCS E Medea Scientific Insitute*

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

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

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

Setting. The study was conducted in a referral center.

Participants. Twelve patients with a genetic diagnosis of FRDA were recruited. Eleven patients completed the trial and 1 patient withdrew from the trial.

Intervention. 11 FRDA patients were treated with IFNy administered subcutaneously at 200 μg x 3 times/week.

Main Outcomes and Measures. Primary efficacy outcome was the Scale for Assessment and Rating of Ataxia (SARA) score. Secondary outcomes were disability and quality of life measured by WHO-DAS and SF-36 questionnaires, PBMC frataxin levels by immunoblotting, cardiac structure and function by echoCG and ECG, the structure of the retina by OCT, and the structure and function of the brain by MRI.

Results.IFNy treatment was generally well tolerated. IFNy completely stopped the progression of the SARA score during the 6-months treatment period, compared to the 6 months preceding the treatment ($p < 0.009$). IFNy reduced the thickness of the cardiac interventricular septum and reduced the Sokolow-Lyon index, with a rebound after treatment discontinuation. It enhanced the activation of the left motor cortex during dominant hand movements and affected the activity of three resting state brain networks. Also, the enhanced activation of the right motor cortex, during bilateral hand movements, correlated with SARA score changes. Other measures did not show significant changes.

Conclusions and Relevance. The results point toward the efficacy of IFNy in affecting the progression of the disease. Placebo-controlled clinical studies are necessary to definitively assess the efficacy of IFNy in FRDA.

Trial Registration. ClinicalTrials.gov Identifier: NCT03888664.

Top-Line Results of a Phase 2, Double-Blind Study to Evaluate Efficacy and Tolerability of TAK-831 in Adults with Friedreich Ataxia

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Background
TAK-831 is an inhibitor of D-amino acid oxidase (DAAO) enzyme that decreases D-serine metabolism. D-serine is an N-methyl-D-aspartate (NMDA)-type glutamate receptor co-agonist and a GluR delta2 agonist. DAAO is highly expressed in the cerebellum, a region responsible for motor coordination. TAK-831 increased the D-serine level in the cerebellum in a genetic mouse model (YG8sR) that has GAA repeat expansions in frataxin. Improvements in beam crossing speed were observed after both single and multiple dose administration of TAK-831 3 mg/kg in this mouse model.
Methods
This study was a Phase 2, randomized, double-blind, placebo-controlled, parallel-arm, proof-of-concept study designed to evaluate the efficacy, safety, pharmacodynamic (PD) effects, and pharmacokinetics (PK) of two dose levels of oral TAK-831 in adult subjects with Friedreich ataxia (FA). Sixty-seven subjects were randomized in approximately a 2:1:2 ratio to placebo, TAK-831 75 mg BID, or TAK-831 300 mg BID. Treatment lasted for 12 weeks. Subjects were assessed on the 9-hole peg test (9-HPT) and other efficacy endpoints at weeks 2, 7, and 12. The primary endpoint was change from baseline on the inverse of time to complete the 9-HPT (9-HPT-1) at week 12.
Secondary outcome measures included the modified Friedreich Ataxia Rating Scale neurological examination (mFARS-neuro), of which the total score provides a functional assessment of patients, the Timed 25-Foot Walk (T25FW), and the Activities of Daily Living component of the FARS (FARS ADLs).
The doses of TAK-831 selected for this study were based on PK data from multiple Phase 1 studies including the SRD/MRD, brain target occupancy data from the PET study, CSF D-serine data, and safety data from all Phase 1 studies. The PK/PD modeling analyses showed that the higher dose regimen resulted in steady-state exposures associated with peak target occupancy of DAAO of >90%. The lower dose was chosen to provide at least a 3-fold exposure difference from the higher dose to understand the dose-response relationship and potentially identify the no-effect dose so that development could move directly into the pivotal study.
Results
The 67 randomized patients were well balanced demographically across treatment arms. Overall, the mean age at screening was 31 years, 37 (45%) patients were female, and 66 (99%) identified their race as white. Regarding disease status, 30 (45%) subjects were ambulatory and the mean baseline plasma D-serine was 0.148 µg/mL. Retention in the study was excellent, with 63 (94%) subjects completing study treatment and follow-up.
The study did not show statistically-significant results on the primary endpoint, change from baseline on the 9-HPT-

1 at week 12. The placebo-adjusted change from baseline was -0.00054 (standard error [SE] = 0.000746) on TAK-831 75 mg BID and -0.00069 (SE = 0.000616) and TAK-831 300 mg BID. These non-significant differences are small in magnitude. Note that on the inverted (1/seconds) scale a positive change indicates higher speed. For the change in mFARS-neuro, all treatment arms showed a trend toward less impairment after 12 weeks, but the TAK-831 arms did not statistically separate from placebo. Likewise, the TAK-831 arms did not statistically separate from placebo on the T25FW test or FARS ADLs. Patients in the 75 mg BID and 300 mg BID groups achieved the targeted TAK-831 exposure and the expected D-serine elevation levels in plasma at steady-state.

TAK-831 was safe and well tolerated in the FA population. Overall, 85% of subjects on TAK-831 had at least one adverse event (AE), compared to 93% on placebo. Most AEs were mild in intensity. One subject had an AE of severe flank pain that was deemed unrelated to treatment; the other AEs were mild or moderate. The most frequently reported TEAEs were headache (30%, 29%, and 42%), fall (22%, 7%, 23%), nausea (15%, 7%, 31%), cough (7%, 7%, 19%), oropharyngeal pain (11%, 7%, 15%), fatigue (15%, 0%, 12%), and nasal congestion (4%, 7%, 15%) for the placebo, 75 BID mg, and 300 BID mg groups, respectively. Four subjects (6%) had AEs that led to discontinuation of study drug. There were no serious AEs or deaths.

Conclusions

Based on the results of this study, we conclude that TAK-831 was safe and well tolerated in FA patients but was not effective as a treatment for this indication. We sincerely appreciate the contributions of the FA patients who enabled us to complete this study in an expeditious manner and reach a clear scientific conclusion.

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