ABSTRACT


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Speaker Abstracts

Speaker abstracts:

Molecular Basis of Disease.

The rapid progress in next-generation genetics of ataxias: insights, challenges, and next steps

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Recent next-generation sequencing (NGS) techniques have allowed to identify an expanding number of novel ataxia genes, and to provide a large share of previously undiagnosed ataxia patients with a molecular diagnosis. This progress prompts several new insights, challenges, and next steps.

Insights. Panel and exome sequencing have revised frequency notions in recessive ataxias. ARSACS, SYNE1 and SPG7 (but not Ataxia Teleangiectasia) might be the most common recessive ataxias following Friedreich's Ataxia. These three recessive ataxias might thus now be selected as promising target diseases for natural history, biomarker and treatment studies. In parallel, these NGS techniques have started start to dissolve the classical traditional classification system driven by clinical diagnosis, which might need to be replaced by an approach of dynamic modular phenotyping. For example, boundaries from ataxias to HSPs (see e.g. SPG7) or to epilepsies (see e.g. KCNA2) have become fluid. These disease groups share not only overlapping phenotypes and underlying genes, but also common cellular pathways and disease mechanisms, which in turn might offer shared hubs for targeted across-disease molecular treatments.

Challenges. NGS techniques have also unraveled a problem which might not yet have received sufficient critical appraisal in research and clinical practice: the identification of missense variants of unknown significance in dominant ataxia genes (like e.g. AFG3L2/SCA28, or ITPR1/SCA29), and partly also recessive ataxia genes (e.g. SYNE1). This emphasizes the need of functional confirmation of missense variants in ataxia genes - not just for research purposes, but in fact primarily also for daily clinical diagnosis.

Next steps. Current NGS techniques still solve an - astonishingly uniform - share of only 20-40% ataxia patients across different labs. This indicates the need to establish novel approaches to solve the still unsolved ataxia patients. The following approaches might be particularly promising: (i) trio analysis for de novo mutations (see e.g. ITPR1); (ii) large-scale exome sharing in joint ataxia NGS pipelines for identifying "second families" (e.g. in GENESIS); (iii) mutational burden analysis. Mutational burden analysis have so far been largely reserved to relatively frequent neurodegenerative diseases like ALS, but might also provide a promising approach in ataxias, if we jointly aggregate data-sets in worldwide common ataxia NGS pipeline.

Finally, the genetic progress achieved by ataxia NGS has now to be translated into systematic translational approaches. The genetic stratification of ataxia patients allowed by NGS is indeed the major bridgehead for identifying special causal pathway mechanisms and preparing targeted molecular treatments in ataxias. Multi-center consortia, like e.g. PREPARE, have now started to create a systematic translational pipeline, facilitating all the crucial translational steps from NGS to standardized preclinical trials, FDA-conform outcome measures, and registry-inventoried transnational trial-ready cohorts in genetic ataxias.

E3 ligase RNF126 directly ubiquitinates frataxin, promoting its degradation: identification of a potential therapeutic target for Friedreich Ataxia.

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Introduction: Friedreich ataxia (FRDA) is a severe neurodegenerative genetic condition, currently lacking an adequate therapy, characterized by reduced expression levels of the mitochondrial protein frataxin. Patients live with a reduced and insufficient amount of frataxin protein, therefore the main goal of a specific treatment for FRDA would be to restore physiological frataxin levels. Since frataxin levels are controlled by the ubiquitin-proteasome system, inhibition of the frataxin E3 ligase, the enzyme responsible for frataxin ubiquitination, may represent a strategy to achieve an increase in frataxin levels by preventing its degradation.
Methods: In order to identify the E3 ligase that ubiquitinates frataxin, we have performed a functional screening of a siRNA library targeting more than 600 E3 ligases, to search for genes whose siRNA-mediated suppression would result in an increase in frataxin levels.
Results: Here we report the identification of the RING E3 ligase RNF126 as the enzyme that specifically mediates the ubiquitination of frataxin precursor, targeting it for proteasomal degradation. RNF126 interacts with frataxin precursor and promotes its ubiquitination in a catalytic activity-dependent manner, both in vivo and in vitro. Most importantly, RNF126 depletion results in frataxin accumulation in cells derived from FRDA patients, highlighting the relevance of RNF126 as a new therapeutic target for Friedreich ataxia (Benini et al., 2017).
Conclusions: Taken together, these results indicate that the E3 ligase RNF126 controls frataxin abundance in cells derived from patients and open the way for the development of a specific therapy aimed at inhibiting RNF126-mediated frataxin degradation.

Benini M, Fortuni S, Condo I, Alfedi G, Malisan F, Toschi N, Serio D, Massaro DS, Arcuri G, Testi
R, Rufini A. 2017. E3 Ligase RNF126 Directly Ubiquitinates Frataxin, Promoting Its Degradation: Identification of a Potential Therapeutic Target for Friedreich Ataxia. Cell Rep 18:2007-2017.

Epigenetic silencing in Friedreich ataxia is caused by hypermethylation of the FXN CpG island shore

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Introduction: Friedreich ataxia (FRDA) is caused by an expanded GAA triplet-repeat (GAA-TR) mutation in intron 1 of the FXN gene that results in epigenetic silencing of the FXN promoter. DNA methylation of CpG island shores, regions that flank human gene promoters when they are embedded in CpG islands, is a known mechanism of epigenetic silencing.
Results: Deep sequencing revealed that DNA hypermethylation spreads from the expanded GAA-TR mutation to the FXN CpG island shore. The CpG island shore is hypermethylated in FRDA, but it remains unmethylated in the non-disease state, and becomes unmethylated when the expanded GAA-TR is reverted to the normal size in isogenic cell lines, thus functioning as a FRDA-specific differentially methylated region (FRDA-DMR). The hypermethylated FRDA-DMR was detected in various patient derived cell types, and also in tissues from the humanized mouse model of FRDA that carries an expanded GAA-TR. Analysis of individual DNA molecules revealed a variegated pattern of DNA methylation within the FRDA-DMR, the magnitude and extent of which was dependent on the length of the expanded GAA-TR mutation. Knockdown of DNMT3A in patient-derived cells reduced methylation of the FRDA-DMR and increased levels of FXN transcript, indicating that DNA methylation contributes to FXN epigenetic silencing in FRDA. Furthermore, treatment with 5-aza-2'-deoxycytidine enhanced the ability of a class I histone deacetylase inhibitor that is known to ameliorate epigenetic promoter silencing in FRDA, to further increase FXN transcript levels in patient-derived cells.
Conclusion: Hypermethylation of the FXN CpG island shore plays a key role in epigenetic silencing in FRDA, and is a novel therapeutic target for reactivation of the epigenetically silenced FXN gene.

Transcriptional profiling of isogenic iPS-derived Friedreich's ataxia sensory neurons

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Introduction: Friedreich's ataxia (FRDA) is caused by the transcriptional silencing of the FXN gene and consequent loss of frataxin protein. How the reduced expression of this essential mitochondrial protein leads to neurological and other systemic symptoms in FRDA patients remains unclear. Similarly to other triplet repeat disorders, it is not known why only specific cells types seem to be affected in the disease, namely large sensory neurons and cardiomyocytes. We seek to uncover the gene expression signature due to GAA.TCC repeat expansion in FRDA neuronal cells, that would help explain the FRDA pathophysiology in affected organs.
Methods: The combination of induced pluripotent stem (iPS) cell technology and genome editing techniques offers the unique possibility of addressing these questions in a relevant cell model of the disease, without the confounding effect of different genetic backgrounds. We derived a set of isogenic iPS cell lines that differ only in the length of the GAA.TCC repeats, using "scarless" gene-editing methods (helper-dependent adenovirus mediated homologous recombination) and performed transcriptomic analysis of iPS-derived CNS and PNS neurons by RNA sequencing.
Results: Three datasets were obtained, two from isogenic lines (comparing FRDA and unaffected CNS and PNS neurons) and one using non-isogenic CNS neurons (comparing two FRDA and two unaffected lines). Differential gene expression analysis showed a remarkable overlap among the three datasets in the pathways identified, which include regulation of cell adhesion, neuronal differentiation, synaptic transmission and gated channel activity. Gene co-expression network analysis performed on the nonisogenic dataset identified modules which, in addition, include genes related to metabolism of lipid and lipoprotein and lysosome.
Conclusions: Using isogenic iPS-derived neurons we find that multiple cellular pathways are commonly affected by the loss of frataxin in CNS and PNS neurons

Early cerebellar mitochondrial biogenesis deficits and OXPHOS complex I and II deficiency in the KIKO mouse model of Friedreich ataxia

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Introduction

Friedreich Ataxia (FRDA) is the most common recessive inherited ataxia resulting from deficiency of frataxin, a highly conserved mitochondrial protein crucial for Fe/S cluster formation and ATP production. Frataxin deficiency is associated with mitochondrial dysfunction in FRDA patients and mouse models. However, early mitochondrial pathophysiology in FRDA cerebellum remains elusive.

Methods

Using the frataxin knock-in/knockout (KIKO) mice and KIKO mice carrying a mitoDendra transgene, a mouse model with neurobehavioral deficits analogous to clinical manifestations in FRDA patients, we examined if the mitochondrial biogenesis PGC-1 /NRF1/Tfam pathways and OXPHOS complex are altered in cerebellum of presymptomatic and symptomatic KIKO mice by immunohistochemistry, Western blotting and OXPHOS complex activity microplate assay kits.

Results

In KIKO mice at presymptomatic ages, levels of mitochondrial biogenesis master regulator PGC-1a and its downstream effectors NRF1 and Tfam are significantly decreased in cerebellar homogenates compared with age-matched controls, suggesting early impairment of cerebellar mitochondrial biogenesis pathways. Early mitochondrial deficiency is further supported by significant reduction of mitochondrial markers GRP75 and mitofusin-1 (MFN1) levels and immunoreactivities in cerebellar homogenates and cortex respectively. Furthermore, the levels and number of mitoDendra are significantly decreased in cerebellar cortex of mitoDendra-KIKO mice, confirming cerebellar mitochondrial biogenesis deficits. Moreover, the OXPHOS complex I and II markers and enzyme activities are significantly decreased in cerebellar homogenates, suggesting complex I and II deficiency in cerebellum of presymptomatic KIKO mice.

Conclusions

Our findings identify early cerebellar mitochondrial biogenesis deficits and OXPHOS complex I and II deficiency as a potential mediator of cerebellar dysfunction and ataxia, thereby providing a potential therapeutic target for early intervention in FRDA patients.

Addressing mitochondrial function in a mouse model of Friedreich's Ataxia (FRDA)

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Background. Friedreich's ataxia (FRDA) is an inherited neurodegenerative disease. The mutation consists of a GAA repeat expansion within the FXN gene, which downregulates the levels of frataxin, leading to abnormal mitochondrial iron accumulation, which may in turn cause changes in mitochondrial function. Although, many studies of FRDA patients and mouse models have been conducted in the past two decades, the role of frataxin in mitochondrial pathophysiology remains elusive. Therefore, we have investigated mitochondrial abnormalities in order to understand the role of frataxin in pathophysiology.
Methods. By using confocal microscopy we assessed an array of functional assays to characterize the possible difference inmitochondrial activity between control and FRDA cells. Here we studied mitochondrial membrane potential (Δ m) and its maintenance, mitochondrial NADH redox state, FAD+ pool, and lipid peroxidation in cerebellar granule neurons of FRDA mouse model. The FRDA mouse model used was generated by the Pook laboratory based upon expression of human FXN transgene containing GAA repeat expansions within a mouse frataxin null background.
Results. Our results show that mitochondria are deregulated in FRDA-like neurons, causing a decrease in mitochondrial membrane potential (Δ m) due to an inhibition of Complex I, which is partially compensated by an overactivation of Complex II. This complex activity imbalance leads to ROS generation in both mitochondrial matrix and cytosol, which results in increased lipid peroxidation. Preventing this increase in lipid peroxidation, in neurons, protects against cell death.
Conclusions. This work describes the pathophysiological properties of the mitochondria in neurons from a FRDA mouse model and shows that lipid peroxidation could be an important target for novel therapeutic strategies in FRDA. By establishing that FRDA is another clinical presentation of a group of disorders due to complex I deficiency, this work may shed a light to a common therapeutic pathway amongst several degenerative disorders.

Mitofusin-dependent ER stress mediates degeneration in a Drosophila model of FriedreichÅLs ataxia

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Introduction. Frataxin downregulation is responsible for FriedreichÅLs Ataxia (FRDA), a rare neurodegenerative disorder that currently lacks an effective treatment. Drosophila models of FRDA have been shown to reproduce the main biochemical and physiological features of this disease. A forward genetic screening performed in our lab identified Drosophila mitofusin (Marf) as an important element in the pathology. Marf knockdown completely suppressed locomotor dysfunction, brain vacuolization and lipid accumulation in frataxin-deficient flies. In mammals and flies, Mitofusins play key roles in mitochondrial fusion/fission as partner of Opa1 and Drp1, in mitophagy as substrate of Parkin and in the interphase between mitochondria and endoplasmic reticulum (ER). Our aim was to unravel the rescue mechanism underlying Marf knockdown.

Methods. Using different histological and molecular markers such as p62, ATG8a, LAMP1, Xbp1 and BiP/GRP78, we have studied effects of frataxin knockdown on mitochondrial morphology, mitophagy and ER function and dissected the roles of Marf in our fly FA model.

Results. We have found that frataxin silencing modified mitochondrial morphology, stimulated mitophagy and altered the endoplasmic reticulum (ER) stress response. Remarkably, our results highlighted that the role of Mitofusin in the ER-Mitochondria axis is underpinning the Marf-silencing mediated protection. In agreement, TUDCA, a chemical chaperone that reduces ER stress, was able to partially ameliorate FRDA defects. Defects in the ER stress response are more than a downstream effect of frataxin depletion. They seem to be necessary to disturb lipid homeostasis and trigger cellular degeneration.

Conclusions. Our results might define a new pathological mechanism in FRDA and suggest that mitochondrial dysfunction and ER stress represent a crucial convergence point in the pathology of the disease. This new relationship between mitochondria and ER in FRDA through mitofusin brings new perspectives towards a better understanding of the complete pathological picture in the disease and towards improvements in FRDA therapy.

Translational Models of Disease

Targeting repeat expansion in cellular models of Friedreich's Ataxia

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Expansions of simple repeat sequences lead to several inherited ataxias. Friedreich's ataxia is unique amongst them as it is caused by large expansions of intronic GAA trinucleotide repeats. The number of expanded GAA repeats correlates with the extent of transcription silencing of the FXN gene and consequently with the extent of frataxin deficiency, however, the exact mechanisms leading to repeat expansion and decreased FXN transcription remain unclear. Inhibiting somatic expansions, stimulating contractions of the already expanded GAAs or removing the repeat tract altogether are considered ultimate therapeutic strategies for FRDA that target the primary defect underlying this disorder. Human cell lines derived from FRDA patients ensure the natural genomic and epigenomic context of the pathogenic GAA repeats, and therefore serve as excellent models to elucidate roles of various cis elements and trans acting factors that affect repeat expansions. We employed a set of FRDA and control primary fibroblasts, induced pluripotent stem cells (iPSCs) as well as cardiomyocytes terminally differentiated from the iPSCs to investigate the role of interplay between transcription and replication at the FXN locus on both GAA expansions and transcriptional silencing. Using singlemolecule
analysis we discovered that expanded GAA repeats present a substantial obstacle for the replication machinery at the FXN locus in FRDA cells. We confirmed that aberrant origin activation and the lack of a proper stress response to rescue stalled replication forks in FRDA cells cause an increase in 3'-5' progressing forks, which could enhance repeat expansion and hinder FXN transcription by inducing head-on collisions with RNA polymerases. Unexpectedly, our analyses of the expanded GAA tracts during prolonged culturing of the FRDA iPSCs demonstrated that increasing transcription of the FXN gene through the use of epigenetic modulators stimulated progressive expansions. Taken together, these results indicate that alleviating replication fork stalling and preventing replication/transcription collision rather than exclusively reactivating FXN transcription might be a more optimal therapeutic strategy for FRDA. Finally, as a complementary approach to pharmacologic intervention, we have demonstrated that precise excision of the expanded GAAs reverses epigenetic changes associated with transcriptional silencing and restores frataxin expression to the levels observed in unaffected cells. Importantly, removal of the expanded GAA repeats also corrected the molecular phenotypes of FRDA iPSC-derived neuronal and cardiac cell models.

Understanding Friedreich's ataxia neuropathophysiology using a new conditional neuronal mouse model.

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Friedreich's ataxia (FA), the most common recessive ataxia, is characterized by sensory and spinocerebellar ataxia and hypertrophic cardiomyopathy. Proprioceptive neurons within the dorsal root ganglia (DRG) are one of the primary affected cells in FA patients. FA is caused by reduced levels of frataxin (FXN), an essential mitochondrial protein involved in the biosynthesis of iron-sulfur (Fe-S) clusters. FXN depletion leads to a Fe-S cluster protein deficit, mitochondrial dysfunction, iron dysregulation and cellular dysfunction. The molecular mechanism underlying neuronal degeneration has not been well established. To decipher the pathological mechanisms in proprioceptive neurons, a new conditional neuronal mouse model (cKO), based on the Cre/LoxP technology, was generated, using the Cre Recombinase expressed under the parvalbumin promoter. Parvlb cKO mice present a FXN depletion in DRG proprioceptive neurons, starting at E17.5, in Purkinje cells of the cerebellum at p40 and in interneurons of the brain at 21.5 weeks. Interestingly, we showed that proprioceptive neurons, which represent only 7.5% of the DRG cell population, express between 50 and 70% of the total FXN of the DRG. Moreover, lumbar DRG express more FXN than cervical DRG. Parvlb cKO mice develop a severe and progressive ataxic phenotype assessed by different behavioural tests and a specific decrease of the sensory wave, revealed by electrophysiological studies. At the molecular level, we identified a deficit of a Fe-S protein, the Succinate Dehydrogenase, in proprioceptive neurons and in Purkinje cells, followed by cellular iron dysregulation, in agreement with elements observed in non-neuronal mouse models. To decipher the downstream events following FXN depletion, RNAseq analysis of DRG was performed and an upregulation of genes known to be expressed by sensory neurons following axonal damage (Regeneration Associated Genes) was identified. Further molecular analyses are ongoing to elucidate the mitochondrial and cellular defects in neurons. Understanding FA neuropathophysiology is critical to develop therapeutical strategies and to identify biomarkers that are essential to validate therapeutical approaches such as gene therapy.

Inducible and reversible phenotypes in a novel mouse model of Friedreich's Ataxia

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Friedreich's ataxia (FRDA), the most common inherited ataxia in humans, is caused by recessive mutations that lead to a substantial reduction in the levels of frataxin (FXN), a mitochondrial iron binding protein. FRDA is a multi-system disease, involving multiple neurological, cardiac, and metabolic manifestations whose study would be substantially advanced by animal models that faithfully recapitulate human disease features. We developed an inducible mouse model of Fxn deficiency that enabled us to control the onset, progression and potential rescue of disease phenotypes by the modulation of Fxn levels using RNA interference. We found that systemic knockdown of Fxn in adult mice led to multiple features paralleling those observed in human patients, including electrophysiological, cellular, biochemical and structural phenotypes associated with cardiomyopathy, as well as dorsal root ganglion and retinal neuronal degeneration and reduced axonal size and myelin sheath thickness in the spinal cord. Fxn knockdown mice also exhibited other abnormalities similar to patients, including weight loss, reduced locomotor activity, ataxia, reduced muscular strength, and reduced survival, as well as genome-wide transcriptome changes. The reversibility of knockdown also allowed us to determine to what extent observed phenotypes represent neurodegenerative cell death, or reversible cellular dysfunction. Remarkably, upon restoration of near wild-type FXN levels, we observed significant recovery of function, pathology and associated transcriptomic changes, even after significant motor dysfunction was observed. This inducible model of FRDA is likely to be of broad utility in therapeutic development and in understanding the relative contribution of reversible cellular dysfunction to the devastating phenotypes observed in this condition.

Voluntary running prevents onset of symptomatic Friedreich's ataxia in mice

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Introduction: The most common clinical symptoms of Friedreich's ataxia (FRDA) include ataxia, muscle weakness, type 2 diabetes and heart failure, which are caused by impaired mitochondrial function due to loss of frataxin (FXN) expression. Endurance exercise is the most powerful intervention to promote mitochondrial function; however, the impact of endurance exercise on FRDA has not been studied. Methods: We assessed exercise intolerance (treadmill running), cardiac function (echocardiogram), whole body glucose metabolism (glucose tolerance test) and protein levels of Fxn, mitochondrial respiratory proteins and antioxidant enzymes (western blot analysis) in a mouse model of FRDA (knockout and knock-in of Fxn, KIKO) at 2,4 and 6 months of age. We also investigated the impact of long-term (4 months) endurance exercise (voluntary wheel running) in KIKO mice. Results: KIKO mice showed reduced Fxn protein expression (-49-69%) in the skeletal muscle, heart and liver compared with wild type mice at all ages, but displayed exercise intolerance, glucose intolerance, and moderate cardiac dysfunction only at 6 months. These functional abnormalities are not due to reduced mitochondrial respiratory proteins and antioxidant enzymes, but impaired mitochondrial respiratory function. KIKO mice heart showed increased protein expression in the fibrosis pathway. Importantly, long-term voluntary running completely prevented these abnormalities and the onset of symptoms in the absence of restoration of Fxn protein expression.
Conclusions: KIKO mice recapitulate human FRDA with age-dependent onset of symptoms along with biochemical abnormalities. Long-term endurance exercise starting at a young age can completely prevent the onset of the disease without correcting the defect of Fxn expression in mice. This is the first study to demonstrate a profound protection of endurance exercise in FRDA in mice, raising exciting possibility of effectively prevention of FRDA by endurance exercise.

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Natural History, Biomarkers and Endpoints

Natural history of Friedreich ataxia

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Friedreich ataxia (FRAD) is a chronically progressing neuromuscular disorder starting in childhood, adolescents or young adulthood. It is caused by GAA repeat extensions in the first intron of the FXN gene which encodes the mitochondrial protein frataxin. GAA repeat extensions suppress the transcription of the FXN gene leading to frataxin deficiency. The wellknown
pathophysiology of FRDA allows to identify treatment targets with the aim to stop its progression and modify the course of the disease. To design such studies knowledge of the natural history of the disease, identification of scales and biomarkers which capture the progression of the disease is of uttermost importance. To prospectively and longitudinally study the natural progression of FRDA the European Friedreich Ataxia Consortium for Translational Studies (EFACTS) set-up a registry. The cross-sectional and 2-year longitudinal data of about 600 patients have been published (1, 2). A similar approach has been taken by an American/Australian registry with published data up to a 5-year follow-up. (3). In the EFACTS registry, the Scale for the Assessment and Rating of Ataxia (SARA) and an ADL scale showed the highest sensitivity to monitor disease progression. The annual progression rate for SARA is 0.77 Å} 0.06 (mean Å} SEM) with a standard response mean (SRM) of 0.33 for one-year and 0.55 for two-year follow-up. If patients are limited to an age below 50 years and a SARA lower than 28 the annual progression is 1.18 Å} 0.08 (mean Å} SEM) with a SRM of 0.83. The data also show that FRDA shows slower progression than reported from retrospective data and imply that a study duration of at least 2 years is necessary in interventional studies aiming at disease modification without a direct symptomatic effect of the drug. These natural history data will now allow to design future treatment trials and select certain patient populations with faster disease progression.

References:
1. Reetz K et al. Lancet Neurol. 2015;14(2):174-82.
2. Reetz K et al. Lancet Neurol. 2016;15(13):1346-54.
3. Patel M et al. Annals of Clinical and Translational Neurology. 2016;3(9):684-94.

Detailing the natural history of Friedreich's Ataxia – loss of ambulation in the CCRNFA study

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Introduction - With >900 subjects across 12 centers in the US, Canada and Australia, the Collaborative Clinical Research Network (CCRN) hosts the largest natural history study in Friedreich's Ataxia (FA) to date. Presently, the maximum follow up time is 13y, and the study is continuously recruiting.

Loss of independent ambulation (LoA) is a major event in FA. With a tremendous burden on quality of life, it's relevance for drug development strategy is obvious. Available estimates for age/disease duration of LoA in FA span a broad range1,2,3 and depend highly on characteristics of the respective cohort, due to disease-related diversity.

Methods – To understand the process of LoA in detail, we studied the age reaching relevant thresholds including gait/stance subscales of FARS, walking/falling problems in (ADL/QOL) questionnaires, timed 25-foot walk and disability staging, using time-to-event analyses. The influence of potential biases was explored and background factors evaluated using cox proportional hazards models.

Figure: Subject Years and Survival Distribution Function for LoA in the CCRN-FA Study.

Results – Exemplarily, a subset of 389 subjects (ambulatory, typical onset), report initial mild problems with walking at 14.6y (95%CI 11.6, 18.9, ADL questionnaire), the first requirement for a cane/walker at 19.1y (95%CI, 18.4, 20.2, FARS-gait item) and eventually LoA at 25.7y (95%CI 24.6, 31.7), defined as stage 5 in functional staging for ataxia. The most apparent predictor of progression to LoA remains the age of first ataxic symptoms.

Conclusion – Using data of 610 ambulatory subjects (1990 patient-years), we report the most comprehensive analysis of the process of LoA in FA. Beyond that, the ideal structure of the cohort (i.e. the recruitment strategy) will allow continuously more precise estimates. The CCRN- FA natural history study provides an unprecedented opportunity to guide future design of clinical trials in FRDA and will facilitate the use of LoA as an endpoint.

References:

1 Harding AE, Friedreich's ataxia: a clinical and genetic study of 90 families with an analysis of early diagnostic criteria and intrafamilial clustering of clinical features. Brain 1981.
2 Dürr A, Clinical and genetic abnormalities in patients with Friedreich's Ataxia. NEJM 1996.
3 Parkinson MH, Clinical features of Friedreich's ataxia: classical and atypical phenotypes. J Neurochem 2013.

Longitudinal MRS, MRI and DTI in the spinal cord in Friedreich's Ataxia: 24-month follow-up

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Purpose

There are very few MR data available in the spinal cord in Friedreich's Ataxia (FRDA). Recently, cross-sectional MR data were reported in the spinal cord, showing structural, microstructural and neurochemical changes [1,2]. Here we report the first longitudinal MRS, structural MRI, and diffusion MRI data in the cervical spinal cord of subjects with FRDA.

Methods

Subjects: Twenty-eight patients with FRDA (age 19.0 Å} 7.3 years, 15F, 13M) and 20 healthy age34

and gender-matched controls participated in the study. In addition, 17 patients returned for 12-month follow-up and 10 patients returned for 24-month follow-up. Most patients were at a relatively early stage of the disease (disease duration 5.6 Å} 3.8 years, FARS score 42.7 Å} 10 at baseline).

MRS: Proton MR spectra (TR/TE=5000/28ms, 256 averages) were acquired in the spinal cord at 3 Tesla using a modified semi-LASER sequence [3] from an 8x6x30 mm^3 voxel positioned along the C4-C5 vertebrae. Spectra were quantified with LCModel using water as an internal reference.

DTI: DTI was acquired to cover C2 to C7 using a readout-segmented echo-planar sequence [4] with: TR/TE = 4500/66ms; voxel size = 1.1x1.1x3.3mm^3; iPAT=2; 30 axial slices; 30 diffusion gradients with b-value= 650 s/mm2 and 6 additional b=0 volumes. DTI was acquired in two opposite phase encoding directions (A-P and P-A) and combined to correct for geometric and eddy current distortions [5]. The spinal cord was manually segmented at the C4-C5 level over 10 mm to obtain average values for fractional anisotropy, and axial and radial diffusivity.

Morphometry: MP-RAGE T_1 images were obtained with 1 mm isotropic resolution. Cervical spinal cord was manually segmented with an ellipse on T_1 images using Spineseg [1] to determine average spinal cord area and eccentricity on three contiguous slices at C2-C3 level.

Results

Cross-sectional:

MRS showed significant cross-sectional differences in tNAA (-40%, p<1e-10), mIns (+30%, p<1e-4), and tNAA/mIns ratio (-47%, p<1e-13). DTI showed significant differences in fractional anisotropy (-15%, p<1e-6), mean diffusivity (+15%, p<0.0005), axial diffusivity (AD, +6%, p=0.05) and radial diffusivity (RD, +25%, p<1e-5). Morphometry showed reduced spinal cord area (-28%, p<1e-7) and increased eccentricity in FRDA (+13%, p<1e-6), consistent with atrophy of dorsal and lateral columns of the spinal cord.

Longitudinal (24-month follow-up):

The tNAA/mIns ratio decreased by 17% on average over 24 months (p=0.02). Fractional anisotropy decreased by 11% (p<0.005) and mean diffusivity increased by 26% (p<0.0005). Spinal cord area decreased by 18% over 24 months (p < 0.0001) and eccentricity increased by 3% over 24 months (p<0.01). Similar trends were already apparent at 12 months, with p-values in the 0.01-0.1 range.

Conclusion

Even though MR in the spinal cord is technically more challenging than in the brain, we detected significant longitudinal alterations in the cervical spinal cord of patients with FRDA at 12 and 24 months. With multiple therapeutic trials currently being planned in FRDA, including gene therapy trials, these data support a role for MRS and MRI as potential markers to assess therapeutic efficacy in clinical trials.

Ackowledgements

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References

[1] Chevis, C.F., et al. Spinal cord atrophy correlates with disability in Friedreich's ataxia. Cerebellum, 2013. 12(1):43-7 [2] Henry P.G. et al. MRS and diffusion MRI of the spinal cord in Friedreich's Ataxia. Proc ISMRM 2014, 571 [3] .z, G.,Tk.č, I., Short-echo, single-shot, fullintensity proton magnetic resonance spectroscopy for neurochemical profiling at 4 T: validation in the cerebellum and brainstem. Magn Reson Med, 2011. 65(4):901-10 [4] Porter, D.A., Heidemann, R.M., High resolution diffusion-weighted imaging using readout-segmented echo-planar imaging, parallel imaging and a two-dimensional navigator-based reacquisition. Magn Reson Med, 2009. 62(2):468-75 [5] Andersson, J. et al. A comprehensive Gaussian Process framework for correcting distortions and movements in diffusion images, Proc ISMRM 2012

Cortical responses and change detection to auditory and somatosensory stimuli in Friedreich ataxia

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Introduction

Neurophysiological assessment of the proprioceptive and cerebellar systems, whose dysfunction and degeneration underlie the afferent and cerebellar ataxia characterizing Friedreich ataxia (FRDA), may define the severity and timing of their involvement, guide the identification of therapeutic targets, and provide biomarkers reflecting disease status, progression and response to treatments.

Previous studies on evoked potentials (EPs) in FRDA proposed that impairment in somatosensory EPs (SSEPs) correlates with GAA1 and does not change with disease progression, while impairment in brainstem auditory EPs (BAEPs) was reported to correlate with disease duration, suggesting an early and stable deficit in somatosensory processing and a progressive involvement of the auditory system. However, a limitation of SSEP studies is the complete loss of these responses in most FRDA subjects, even at a young age, a finding we could confirm with our patients.

Methods

We used magnetoencephalography (MEG) to study cortical evoked responses of FRDA subjects to somatosensory and auditory stimuli, with the assumption that this technology might allow to detect responses even when traditional EPs cannot be measured, and add temporal and spatial resolution to the analysis. We also explored MEG signals generated by sensory change detection, which are thought to be modulated by the cerebellum. In traditional EP protocols, an increased evoked response, called mismatch negativity (MMN), is observed when a deviant stimulus occurs amongst a sequence of repeated standard stimuli. MMN is the correlate of pre-attentional change detection in sensory cortices. An equivalent signal can be measured by MEG. Of notice, unilateral cerebellar lesions lead to near absent MMN for ipsilateral deviant somatosensory stimuli, but have no effect on auditory change detection.

We studied 16 FRDA patients (10 females, 6 males), with a mean age of 30 years (range 9-53) and a mean SARA score of 23.4 (range 9.5-37.5), and 16 healthy controls (9 females, 7 males), with a mean age of 29 years (range 10-55). We recorded whole-scalp MEG (Elekta, Oy) while undergoing (1) a tactile oddball paradigm where standard stimuli consisted of pneumatic stimulation of the right forefinger fingertip and deviant stimuli of simultaneous stimulation of the first two phalanxes; and (2) a monaural auditory oddball paradigm where standard stimuli consisted of audible tones of 540 Hz and deviant stimuli were 600 Hz tones, presented in the right hear. Inverse modelling was done using the Minimum Norm Estimate (MNE). For group analysis, individual source power time series were normalized by the maximum amplitude of standards responses before group averaging, to exclude individual subjects' amplitude effect. We temporally realigned time series on the first peak activation to control for individual responses latencies. We used non-parametric permutation statistic tests to assess significance of evoked responses.

Results

Cortical somatosensory evoked responses were found in all subjects at left primary somatosensory cortex (SI). In FRDA subjects their mean latency was significantly longer (53 vs 28 ms; $p<0.001$), and their mean amplitude was significantly smaller (0.285 vs 0.513; $p=0.0041$) than in controls. GAA1 negatively correlated with the amplitude of individual SI responses ($r=-0.74$, $p=0,0032$). Cortical auditory evoked responses were found in all subjects at primary auditory cortex (AI), bilaterally. In FRDA patients their mean latency was significantly longer than in controls (107 vs 87 ms; $p<0.001$), but their amplitude was comparable in FRDA and

controls (0.507 vs 0.45; p=0.25). GAA1 negatively correlated with individual AI responses (r=-0.56, p=0.036) of FRDA subjects. Larger amplitude responses to deviant stimuli, the MEG equivalent of MMN, were found in controls and FRDA patients at left secondary somatosensory cortex (S2), with a delay of 100-200 ms; but also at left S1 in FRDA patients, with a delay of 50-78ms.
The normalized magnitude of deviant stimuli responses was significantly smaller for FRDA patients, and negatively correlated with GAA1 (r=-0.6, p=0.023). Similarly, responses to deviant auditory stimuli were found in patients and controls over the left superior temporal lobe, with a delay between 150-200ms and comparable normalized magnitudes for both groups.
Conclusions
MEG allows to detect cortical responses to tactile stimuli in all FRDA patients, even when SSEPs are absent. These responses are delayed and reduced in amplitude. Cortical auditory responses are not decreased in amplitude, but show increased latency. In both cases, impairment is seemingly unrelated to disease progression and only correlates with mutation severity, indicating that these parameters are biomarkers of early sensory damage. Cortical responses to deviant somatosensory stimuli (corresponding to MMN) are normally measured at S2 only, as it was the case with our controls, but in FRDA subjects they occurred in S1 as well.

Exercise stress testing on adaptive equipment is feasible and reliable in Friedreich Ataxia

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Introduction: Outcome measures which reflect clinically relevant changes in neurologic and cardiac function are needed in Friedreich Ataxia (FRDA). Traditional exercise equipment is not suited for most patients with significant gait and balance problems. We aimed to test whether exercise stress test (EST) performance on adaptive equipment is feasible and reliable in FRDA patients.

Methods: Subjects with genetically confirmed FRDA underwent incremental cardiopulmonary exercise testing on either an arm (ACE) or recumbent leg (RLCE) cycle ergometer at up to 4 visits (baseline, 2 wks, 4 wks, 1 yr). The ramp protocol was continued while subjects were advised to pedal at a constant rate until maximal volition or until limiting symptoms occurred. Maximum work rate, oxygen consumption (VO2max), oxygen (O2) pulse, and anaerobic threshold (AT) were ascertained. Test-retest reliability was assessed by intraclass coefficient (ICC) from visits 2 and 3.

Results: 23 subjects enrolled with mean FARS 59 Å} 16, age 19 Å} 8 yrs, age of onset 9 Å} 4 yrs, GAA1 741 Å} 195 (except 2 with G130V point mutations). 21 (91%) completed a maximal EST; 2 subjects (FARS 82 and 89, GAA1 900 for both) could not keep a steady cadence to reach maximal volition on either ACE or RLCE attempts. ICC for work rate was excellent for ACE [0.98 (95%CI 0.86,0.998)] and RLCE [0.97(0.76,0.997)], while ICC was low on other parameters for ACE but high for RLCE: O2 pulse 0.94 (0.52, 0.993), VO2max 0.93 (0.47, 0.992), AT 0.96 (0.69, 0.996).

Conclusions: Maximal EST on adaptive equipment is feasible for FRDA patients with high testretest
reliability. Clinical trials enrolling subjects who can complete arm cycle ergometry testing should consider maximum work as an outcome measure, while trials enrolling subjects who can complete recumbent leg ergometry testing can also use VO2max, O2 pulse and AT as outcome measures.

Developing a clinically meaningful instrumented measure of upper limb function in Friedreich ataxia.

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Introduction: Friedreich ataxia (FRDA) has a significant effect on upper limb function which in turn, compromises independence and quality of life. The most common measure of upper limb function in FRDA is the Nine Hole Peg Test (9HPT). Increasingly, regulatory bodies are calling for outcome measures to reflect changes in functional status however the capacity for the 9HPT to reflect functional capacity is uncertain. The aims of this study were twofold: 1) to identify the functional upper limb tasks that individuals with FRDA found most challenging and 2) and use these results, to develop and pilot an instrumented measure of upper limb function that captures burden of disease and potentially clinically meaningful change.
Methods: We analysed the upper limb component of the Friedreich Ataxia Impact Scale (FAIS) in 120 individuals with FRDA. In addition, we examined performance on the Jebsen Taylor Hand Function Test (JHFT) and 9HPT in 73 individuals with FRDA correlating both measures with clinical parameters of FRDA. Based on this analysis we developed an instrumented motion capture functional upper limb measure for FRDA.
Results: Intricate tasks such as taking a spoon to the mouth proved to be most problematic in 88% of participants, significantly correlating with age at disease onset ($r=-0.229$, $p<0.05$), disease duration ($r=0.53$, $p<0.00$), the dominant 9HPT ($r=0.37$, $p<0.00$) and all items in the upper limb section of the Friedreich Ataxia Rating Scale (FARS). Simulated feeding with the dominant hand on the JHFT significantly correlated with disease duration ($rho=0.40$, $p<0.00$) and the 9HPT ($rho=0.58$, $p<0.00$).
Conclusion: We have systematically identified a functional task that has provided the genesis for development of a true measure of upper limb function. This novel instrumented measure aims to accurately reflect upper limb function in individuals with FRDA and as such will be of significant utility in future clinical trials.

Cardiac magnetic resonance T1 mapping as a window into the myocardium in Friedreich ataxia (FRDA)

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Introduction: Left ventricular (LV) concentric remodeling or hypertrophy is common in FRDA and is associated with poorer prognosis. However, the nature of the underlying myocardial ultrastructural change in FRDA is unknown. The aim of this study was to investigate cardiac magnetic resonance (CMR) T1 mapping, a technique used to assess the fibrous tissue burden of the myocardium.

Methods: A standardized CMR protocol including T1 mapping and measurements of LV mass, LV end- diastolic volume, the native T1 time, post gadolinium (PostGad) T1 time, calculation of the LV mass/volume ratio (LVMVR) and the partition coefficient (PC) was performed on individuals from Melbourne and Philadelphia who were homozygous for GAA repeats in the FXN gene.

Results: Fifty-seven subjects (55% males) with FRDA were recruited from both sites; the median (range) age was 24 (10-51) years, age at onset 10 (2-28) years, disease duration 11 (3-36) years and GAA1 repeat length 694 (241-1050). Four subjects had an LV ejection fraction (EF) <55% and 31 had an increased LVMVR (>1.20). The native T1 time was similar in both cohorts, but the Philadelphia cohort was younger and had a higher PostGad T1 time and PC than the Melbourne cohort ($p<0.01$ for all). On multivariate analysis, after adjusting for sex, age and site, there were positive correlations of GAA1 with native T1 time ($b=0.47$, $p=0.001$) and PC ($b=0.52$, $p<0.001$), but no relation of GAA1 with PostGad T1 time. LVMVR was also positively correlated with native T1 ($b=0.30$, $p<0.02$), but when LVMVR was combined with GAA1, only GAA1 remained a significant correlate of native T1.

Conclusion: T1 mapping demonstrates positive correlation of myocardial fibrous burden with GAA1, and provides information about the LV myocardium in FRDA which is independent of the presence of LV remodeling/hypertrophy. Information is required regarding the relationship of T1 variables with cardiac outcomes

Therapeutics and Clinical Trials

Summary and lessons learned from ataxia trials

Francesco Sacca

Innovative trial designs for rare diseases, with focus on use of innovative endpoints and potential use of registry data.

Prof Dr Kit CB Roes
University Medical Center Utrecht

In clinical trials that aim to provide confirmatory evidence for new treatments for rare diseases, the available sample size is often the crucial limitation. In a regulatory (drug approval) setting decision making based on evidence from a limited number of small trials is challenging. The totality of evidence is commonly taken into account, although in an informal fashion. To improve underlying methods as well as decision making our research followed different pathways, amongst which (1) how to optimize methods for (flexible) designs in case of finite and small sample sizes, (2) appropriate methods for meta-analysis of small number of small trials to support decision making and (3) investigate new patient centered outcomes that capture the often heterogeneous disease course efficiently (such as mitochondrial diseases, Duchenne's muscular dystrophy, e.g.). A brief general overview of potential methods will be given, with subsequent focus on development of individualized outcomes, goal attainment scaling, and the use of registry data as potential basis for (historical control) data and to better design clinical trials.

Activation of Frataxin expression by duplex RNAs and antisense oligonucleotides

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Friedreich's Ataxia (FRDA) is an incurable genetic disorder caused by a mutant expansion of the trinucleotide GAA within an intronic FXN RNA. This expansion leads to reduced expression of frataxin (FXN) protein and evidence suggests that transcriptional repression is caused by an R-loop that forms between the expanded repeat RNA and complementary genomic DNA. Synthetic agents that increase levels of FXN protein might alleviate the disease. We demonstrate that introducing anti-GAA duplex RNAs or single-stranded locked nucleic acids (LNAs) into patient-derived cells increases FXN protein expression to levels similar to analogous wild-type cells. Our data are significant because synthetic nucleic acids that target GAA repeats can be lead compounds for restoring curative FXN levels. Both antisense oligonucleotides (ASOs) and duplex RNAs (dsRNAs) were shown to activate FXN expression, providing two starting points for therapeutic development. Our results demonstrate that interfering with R-loop formation can trigger gene activation and reveal a new strategy for up-regulating gene expression. Current studies are focusing on exploring how chemical modifications affect the potency of activation. We are also examining the potency of activation in patient-derived cell lines that contain diverse numbers of mutant repeats. The purpose of this research is to identify the best compounds for animal studies and the prospects for these studies will be evaluated. The outstanding question is whether the promising results in cell culture can be translated successfully in vivo.

Gene-targeted synthetic molecules stimulate transcription through repressive GAA-repeats in patient-derived Friedreich's Ataxia cells

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Regulated release of paused RNA polymerase II (Pol II) into productively elongating state is a critical rate-limiting step in the expression of genes involved in development, differentiation and disease. Based on recent mechanistic insights, we designed molecules that function as synthetic transcription factors at targeted genomic loci. We apply this design to generate a molecule that enables transcriptional elongation through GAA microsatellites that silence Frataxin expression and lead to incurable Friedreich's ataxia. The molecule restores expression in primary cells from multiple patients bearing a range of GAA-repeat expansions. Our portable design provides a framework to generate a class of molecules that license Pol II to overcome repeat-induced repressive barriers to transcription elongation at other genomic loci.

Class-I HDAC inhibitors with improved potency and drug-like properties for derepressing frataxin production in Friedreich's Ataxia

Shripad S. Bhagwat, Helen Hua, Greg Luedtke, Alex Bridges, Michael Robinson, Doug Boatman, Elizabeth Soragni, Joel Gottesfeld, David Jacoby

Friedreich's Ataxia (FA) is a fatal genetic disease caused by production of insufficient amounts of frataxin protein in humans. Release of frataxin (FXN) gene silencing is a promising approach to treat FA. Class I histone deacetylase (HDAC) inhibitors that have a characteristic amino-benzamide as the substructure that binds zinc in the active site of HDAC, are found to be effective in cellular and animal models of FA. One such drug, RGFP109, was found to increase frataxin after a single oral dose in humans. However, 109 was discontinued from clinical development because of metabolic liabilities.

BioMarin has evaluated a large number of the amino-benzamide class of HDAC inhibitors in order to identify compounds that have increased potency for upregulating FXN expression, increased drug exposure in the disease specific tissues (brain and heart), a longer half-life in the body, and lower potential to form the undesired metabolites. An update on our search for an improved compound for the treatment of FA will be discussed during the presentation.

RNA/DNA hybrid interactome uncovers DHX9 as a novel regulator of pathological R-loops in Friedreich ataxia

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Friedreich ataxia (FRDA) is the most common inherited recessive ataxia, characterised by progressive sensory ataxia, cardiomyopathy, diabetes and premature death. It is caused by an expanded GAA repeat sequence in intron 1 of the frataxin (FXN) gene, resulting in frataxin mRNA and protein deficiency. Using methodology established in the lab (Skourti-Stathaki et al., 2011), we recently demonstrated that FXN silencing involves formation of unusual RNA/DNA structures, R-loops, over the expanded repeats (Groh et al., 2014). These R-loops are stable and trigger formation of repressive chromatin marks over the FXN gene. To elucidate the molecular mechanism of R-loop-mediated transcriptional silencing in FRDA, we designed an affinity purification approach coupled to mass spectrometry to identify R-loop-binding proteins (R-loop interactome) in an unbiased way. Based on this approach, the R-loop interactome consists of known R-loop factors SRSF1, FACT and Top1 and yet uncharacterised interactors, including RNA-/DNA-binding proteins, DNA repair and chromatin factors, and helicases. We investigated the function of the top R-loop interactome candidate, helicase DHX9, in vivo. We found that DHX9 recruitment to FXN gene is compromised in FRDA cells, resulting in increased R-loop accumulation over the expanded repeats. Interestingly, DHX9 overexpression promoted the resolution of diseaseassociated R-loops. These data suggest that DHX9 represents a novel regulator controlling R-loop metabolism in FRDA disease. Thus, RNA/DNA hybrid interactome provides a powerful resource to study R-loop biology in health and disease and reveals potential targets for future therapeutic interventions of FRDA.

Skourti-Stathaki K, Proudfoot NJ, Gromak N. 2011. Human senataxin resolves RNA/DNA hybrids formed at transcriptional pause sites to promote Xrn2-dependent termination. Molecular cell 42: 794-805.
Groh M, Lufino MM, Wade-Martins R, Gromak N. 2014. R-loops associated with triplet repeat expansions promote gene silencing in Friedreich ataxia and fragile X syndrome. PLoS genetics 10: e1004318.

Safety, Efficacy, and Pharmacodynamics of Omaveloxolone in Friedreich's Ataxia Patients (MOXIe Trial): Part 1 Results

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INTRODUCTION:
Previous studies have demonstrated that suppression of Nrf2 in Friedreich's ataxia patients contributes to excess oxidative stress, mitochondrial dysfunction, and reduced ATP production. Omaveloxolone, an Nrf2 activator and NF-kB suppressor, targets dysfunctional inflammatory, metabolic, and bioenergetic pathways. The initial dose-ranging portion of a Phase 2 study of the safety, efficacy, and pharmacodynamics of omaveloxolone in Friedreich's ataxia patients (MOXIe, NCT02255435) sought to evaluate the optimal omaveloxolone dose for further study.
METHODS:
Sixty-nine Friedreich's ataxia patients were randomized 3:1 to either omaveloxolone or placebo administered once daily for 12 weeks. Patients were randomized in cohorts of 8 patients, at dose levels of 2.5, 5, 10, 20, 40, 80, 160, and 300 mg. Eligible Friedreich's ataxia patients must have had a modified Friedreich's Ataxia Rating Scale (mFARS) score $\geq$10 and $\leq$80, be between 16 to 40 years of age (inclusive), and be able to complete maximal exercise testing.
RESULTS:
Optimal pharmacodynamic and efficacy changes were observed at omaveloxolone doses of 80 and 160 mg. At the 160mg dose, omaveloxolone improved mFARS by 3.8 points versus baseline ($p=0.0001$) and by 2.3 points versus placebo ($p=0.06$). Omaveloxolone produced greater improvements in mFARS in patients that did not have preexisting musculoskeletal foot deformity (pes cavus). In patients without this foot deformity at the 160mg dose level, omaveloxolone improved mFARS by 6.0 points from baseline ($p<0.0001$) and by 4.4 points versus placebo ($p=0.01$). Omaveloxolone was well tolerated, and adverse events were generally mild in severity.
CONCLUSIONS: Treatment of Friedreich's ataxia patients with omaveloxolone at the optimal dose level led to improvements in neurological function (mFARS). Therefore, omaveloxolone treatment will be examined in greater detail at the optimal dose level in Part 2 of the MOXIe study.

Lessons learned from recent approvals of therapies for neuromuscular disorders.

Jane Larkindale, Friedreich's Ataxia Research Alliance and Duchenne Regulatory Science Consortium, Critical Path Institute.

Several drugs for neuromuscular disorders have been approved by the US Food and Drug Administration (FDA) and/or the European Medicines Authority (EMA) in recent years. These include Spinraza for spinal muscular atrophy (SMA), Exondys51, Emflaza and Translarna for Duchenne Muscular Dystrophy and Radicava for Amyotrophic Lateral Sclerosis (ALS). Each drug has taken a different path to approval: several are approved by only one authority and several have utilized accelerated approval pathways. Trials have varied significantly in size and design, and many protocols have utilized biomarkers and natural history data to support their cases for approval. Many of these therapies have generated controversy around their supportive data, the populations of patients they should be approved for and around the pricing of such therapies. This talk will review the work that led to approval of these therapies and lessons learned from those approvals that can be applied to future studies of potential therapeutics for ataxias.

Overview of viral gene therapy approaches for genetic diseases

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We now have over 30 years of experience with a variety of gene therapy vectors for the correction of genetic diseases. Although we have yet to see a successful gene therapy drug enter the marketplace, two gene delivery systems have proven to be capable of efficient delivery of genes to somatic cells in animal models. Both are capable of long-term correction of disease symptoms in animal models and in humans. These are Adenoassociated virus (AAV) and retroviruses. We will compare the biology of these two delivery systems, with a particular focus on AAV. Safety issues, the current production and downstream processing issues associated with these vectors and quality control issues that need to be considered will be outlined. We will also discuss the routes of administration that can be used for both peripheral (systemic) and CNS delivery. In addition, we will briefly discuss the immune response to these vectors and methods that are being tried to minimize the immune response. Finally, we will outline approaches that are currently being used to develop the next generation of vectors, which promise to be more efficient and more cell and organ specific. These vectors are likely to deliver not just replacement genes, but also elements that knock down endogenous genes (miRNA), site specific endonucleases that modify mutant genes or constructs that correct mutant genes by homologous recombination.

Neurotrophic factor and cytokine mimetics as new potential therapeutic agents for Friedreich's ataxia

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Friedreich's ataxia (FRDA) is a neurodegenerative disease caused by recessive mutations that produce a deficiency of frataxin. Research in our lab and others has demonstrated that neurotrophic factors like Brain-Derived Neurotrophic Factor (BDNF) and cytokines like Erythropoietin (Epo) can prevent neurodegeneration triggered by frataxin deficit and increase frataxin expression in different experimental models. However the therapeutic application of these protein factors is seriously limited because of their poor pharmacokinetics and their inability to effectively cross the blood brain barrier. In order to address these limitations, we have explored the therapeutic potential of smaller molecules which can activate BDNF or Epo receptors.

One of these molecules, 7,8-dyhydroxyflavone (7,8-DHF) is known to activate TrkB BDNF receptors. Our results indicate that 7,8-DHF decreases the levels of apoptotic markers and increases frataxin expression in primary neuronal cultures from both wild-type and FRDA model YG8 mice. Moreover 7,8-DHF also leads to a significant increase in frataxin in cultures of FRDA patient- derived olfactory mucosa stem cells. Interestingly, the systemic administration of 7,8-DHF to YG8 mice in vivo enhances frataxin expression in the cerebellum but not in the heart of treated animals (which is consistent with the higher expression of TrkB in the cerebellum). Another molecule, the helix B surface peptide (HBSP), has been reported to activate the cytoprotective Epo receptor without stimulating erythropoiesis. HBSP also increases frataxin expression in primary neuronal cultures from both wild-type and YG8 mice, as well as in FRDA patient-derived olfactory mucosa stem cells. More interestingly, the systemic administration of HBSP to YG8 mice in vivo increases frataxin both in cerebellum and heart of treated animals. In view of these data we suggest that neurotrophic factor and cytokine mimetics such as 7,8-DHF and HBSP should be considered as a novel strategy to increase frataxin expression and delay neurodegeneration in FRDA.

Gene therapy for Friedreich's ataxia

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AAV-mediated gene therapy has gained significant momentum over the past few years with an increasing number of subjects enrolled in clinical studies. In this presentation, I will review the pathway to launching clinical gene therapy studies. Initial studies have been aimed at demonstrating safety in open-label studies using several routes of admiration. New FDA and EMEA guidelines which support product development in rare disease will facilitate the drug development path for gene therapy approaches. These new pathways have been reinforced by the 21st Centuries Cures Act, recently approved legislation in the USA.

In addition to new regulatory pathways, there are opportunities for improved access to clinical trial material and innovative clinical trial design which may be of value in Friedreich's ataxia. Supply of sufficient high quality clinical product is an important part of the clinical study plan. The latest observations from a novel clinical AAV production strategy will be reviewed to establish the basis for sufficient clinical supply to meet broad clinical demand in current and future gene therapy studies.

Lastly, the management of preexisting immune response and the ability to re-dose AAV vectors remains a critical barrier in clinical implementation of AAV-mediated gene therapy. Findings from recent IND-enabling studies will be reviewed to consider the options for management of this issue in FA gene therapy.

Nicotinamide Mononucleotide supplementation in a model of Friedreich's Ataxia cardiomyopathy improves cardiac function and bioenergetics in a SIRT3-dependent manner

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Background/Hypothesis: Increasing NAD+ levels by supplementing with the precursor nicotinamide mononucleotide (NMN) improves cardiac function in multiple mouse models. While NMN influences several aspects of mitochondrial metabolism, the molecular mechanisms by which increased NAD+ enhances cardiac function are poorly understood. A putative mechanism of NAD+ therapeutic action is via activation of the mitochondrial NAD+-dependent protein deacetylase sirtuin 3 (SIRT3). We assessed the therapeutic efficacy of NMN and the role of SIRT3 in the Friedreich's Ataxia cardiomyopathy mouse model (FXNKO).
Methods/Results: At baseline, the FXNKO heart has mitochondrial protein hyperacetylation, reduced SIRT3 mRNA expression, and increased demand for NAD+. Remarkably, NMN administered to FXNKO mice restored cardiac function to levels near normal. To determine whether SIRT3 is required for NMN therapeutic efficacy, we generated SIRT3KO and SIRT3KO/FXNKO (dKO) knockout models. The improvement in cardiac function upon NMN treatment in the FXNKO is lost in the dKO model, demonstrating that the effects of NMN are dependent upon cardiac SIRT3. Coupled with cardioprotection, SIRT3 mediates NMN-induced improvements in both cardiac and extracardiac metabolic function and energy metabolism.
Conclusions: Taken together, these results serve as important preclinical data for NMN supplementation or SIRT3 activator therapy in Friedreich's Ataxia patients.

Correction of sensory ataxia in a novel mouse model of Friedreich ataxia using gene therapy approach

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Friedreich's ataxia (FA), the most common autosomal recessive ataxia, is characterized by a sensory and spinocerebellar ataxia, hypertrophic cardiomyopathy and increase incidence of diabetes. FA is caused by reduced levels of frataxin (FXN), an essential mitochondrial protein involved in the biosynthesis of iron-sulfur (Fe-S) clusters. Impaired mitochondrial oxidative phosphorylation, bioenergetics imbalance, deficit of Fe-S cluster enzymes and mitochondrial iron overload occur in individuals with FA. Proprioceptive neurons within the dorsal root ganglia (DRG) and cardiomyocytes are the most affected tissues in FA patients. To date there are not effective treatment for FA.

We have previously established the primary proof-of-concept for developing gene therapy of FA cardiomyopathy and showed that adeno-associated virus (AAV) rh.10 vector expressing human FXN injected intravenously rapidly and completely reversed the cardiac disease (Perdomini et al, 2014). We recently generated a novel mouse model that recapitulates faithfully the sensory ataxia associated to FA using the conditional approach to delete frataxin specifically in the parvalbumin expressing cells, including the proprioceptive neurons of the DRG. Using this mouse model, we have developed an AAV gene therapy approach based on an intravenous delivery of AAV9-CAG-hFXN-HA vector and shown at an early symptomatic stage of the disease a complete prevention of the ataxic phenotype and electrophysiological analysis showed maintenance of the sensory wave in the treated animals. Histological studies revealed a complete prevention of neuronal loss in the DRG.

We then evaluated the therapeutical approach at a post symptomatic stage of the disease with a combination of an intravenous delivery of AAV9-CAG-hFXN-HA vector and 3 intracerebral deliveries of AAVrh10-CAG-hFXN-HA. Treated animals displayed a complete reversion of the proprioceptive phenotype evaluated by gait analysis, coordination test and EMG as well at the histological levels with a full preservation of neurons within DRG and a complete regeneration of axons within peripheral nerves. Together, our results provid a proof-of-concept for developing gene therapy for the sensory ataxia in FA.

Phenotypic and functional characterization of sensory neurons derived from human pluripotent stem cells and examining their in vivo capability to integrate into adult dorsal root ganglia.

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Introduction: Friedreich ataxia (FRDA) is a disease characterised by neurodegeneration and cardiomyopathy. FRDA is due to insufficiency of the mitochondrial protein, FRATAXIN, which leads to mitochondrial dysfunction, cell toxicity and cell death, particularly within the nervous system and cardiac tissue. The sensory dorsal root ganglia (DRG) is one of the primary and most significant sites of degeneration occurring in FRDA. Sensory neurons derived from human pluripotent stem cells (hPSC) are a valuable resource to develop regenerative therapies to treat FRDA either for drug discovery platforms and/or cell replacement transplants.
Methods: We have developed an efficient system for deriving DRG sensory neurons from hPSC. Here we phenotypically and functionally characterize the hPSC-derived sensory neuronal subtypes using Q-PCR, immunostaining and multi-electrode array analyses. Furthermore, hPSC-derived sensory neurons were transplanted in the adult rat DRG regions to examine their capacity to mature and functionally integrate in vivo.
Results: Our data shows that hPSC-derived cultures consist of heterogeneous population of sensory neuronal subtypes, including proprioceptors, mechanoreceptors and nociceptors. We demonstrate their functionality in vitro using multi-electrode arrays and also their ability to mature and integrate in vivo when transplanted into the adult DRG region. Some in vitro and in vivo analyses have also been performed using FRDA induced pluripotent stem cells (iPSC).
Conclusions: These studies show promising outcomes for using FRDA iPSC to treat peripheral sensory neurodegeneration.

Intravenous delivery of AAV gene therapy to cerebellum and peripheral tissues critical for the treatment of Friedreich's ataxia.

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Introduction
Adeno-associated viral (AAV) vectors have great potential for therapeutic gene delivery. A major challenge of AAV gene therapy is delivering the transgene to target cells at levels that result in expression that is safe and effective. In larger mammals including primates, only relatively limited gene transfer to the adult CNS has been achieved to-date following systemic administration. For FA, primary sites of pathology are the dentate nucleus of the cerebellum, dorsal root ganglia (DRG) and heart. Here, we describe studies with novel AAV capsids in non- human primates to evaluate the potential of intravenous (IV) administration for frataxin gene delivery to the cerebellum, PNS and peripheral organs for the treatment of FA.
Methods
AAV vectors comprising novel capsids and a HA-tagged frataxin transgene were administered IV to non-human primates and approximately 1 month later, frataxin-HA gene transfer to target tissues was assessed. Bio-distribution and cellular tropism were evaluated by HA-tag immunohistochemistry, whereas vector genome levels were quantified with digital PCR. Frataxin-HA levels were determined by ELISA.
Results
Significant gene transfer to the dentate nucleus of the cerebellum was observed. Staining for the HA-tag labelled considerable numbers of neurons. Gene transfer was also high to DRG and heart, and accompanied by high exogenous frataxin expression evident by ELISA and HA- staining.
Conclusions
Studies in the non-human primate support intravenous dosing with a novel capsid as a potential approach for the treatment of central and peripheral FA with AAV gene therapy.

Effects of acetyl-DL-leucine in cerebellar ataxias

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Background. Acetyl-DL-leucine (AL) is a modified amino acid which has been used to treat vertigo since 1957. It may act due to its direct effect on neurons, as was shown in the vestibular nuclei. Due to the phylogenetical and electrophysiological similarities and close interactions between vestibular and deep cerebellar neurons, we had hypothesized that there may also be a positive effect on ataxic symptoms in cerebellar disorders.

Results. In 2013, in a first case series on 13 patients with different types of cerebellar ataxia we showed that AL (5 g/ day for 1 week) significantly improved the symptoms in terms of SARA, SCAFI and Qo [4]. Mean total SARA (Å} SD) decreased from a baseline of 16.1Å}7.1 to 12.8Å}6.8 on medication (p = 0.002). There were also significant improvements in sub-scores for gait, speech, finger-chase, nose-finger-test, rapid alternating movements and heel-toshin. Furthermore, patients showed a significantly better performance in the SCAFI consisting of the 8-m-walking-time, 9HPTD and the PATA rate. QoL increased during treatment (p = 0.003). No side effects were reported. (Videos: www.dgn.org)).

In 2015 we reported in a case series on 12 patients with Niemann-Pick type C (NPC) that AL (5 g per day for one month with a one-month titration) significantly improved the clinical symptoms, measured by SARA, SCAFI, modified disability rating scale (mDRS) and EQ-5D-5L Quality of Life [1]. The total SARA-score changed significantly from a baseline of 10.8Å}11.2 to 7.0Å}10.7 after one month on medication and 10.5Å}11.5 post 1 month of washout, indicating an improvement of cerebellar signs on medication (p = 0.000412). The total mDRS score was 10.0Å}5.35 at baseline, 9.0Å}5.3, on medication and 10.0Å}5.4 after one month of washout. The 9HPTD changed significantly on medication. In terms of QoL, the visual analog scale of EQ-5D-5L also changed significantly on medication (videos: www.neurology.org).

A third case series demonstrated an improved so-called coefficient of variation of stride time in the gait analysis of 14 patients with cerebellar ataxia during a treatment with AL [3]. The improvement of variability was restricted to the condition of slow walking, where walking stability is thought to critically rely on the sensory integration function of the cerebellum. It should be mentioned that in another case series with 10 patients with degenerative cerebellar ataxia, no improvement in SARA was observed [2]. However, 7 out of 10 patients described a subjective improvement on medication.

Conclusions and limitations. Acetyl-DL-leucine significantly improved ataxic symptoms without side effects and therefore showed a good risk-benefit profile. The added value of the above-mentioned case series is the demonstrated safety and tolerability of the agent in various medical conditions with the common symptom of cerebellar ataxia. The obvious limitations of these studies are a) the lack of reference agent (placebo), b) the non-blinded design, and c) the small sample size.

Reference List

1. Bremova T, Malinova V, Amraoui Y, Mengel E, Reinke J, Kolnikova M, Strupp M (2015) Acetyl- dl-leucine in Niemann-Pick type C: A case series. Neurology 85:1368-1375
2. Pelz JO, Fricke C, Saur D, Classen J (2015) Failure to confirm benefit of acetyl-DLleucine in degenerative cerebellar ataxia: a case series. J Neurol 262:1373-1375
3. Schniepp R, Strupp M, Wuehr M, Jahn K, Dieterich M, Brandt T, Feil K (2016) Acetyl-DL-leucine improves gait variability in patients with cerebellar ataxia-a case series. Cerebellum Ataxias 3:8
4. Strupp M, Teufel J, Habs M, Feuerecker R, Muth C, van de Warrenburg BP, Klopstock T, Feil K (2013) Effects of acetyl-DL-leucine in patients with cerebellar ataxia: a case series.

Poster Abstracts
POSTER SESSION ONE

Thursday 28 September
h. 5.30 – 7.30 pm

Molecular Basis of Disease

3. Analysis of GAA repeat interruptions in a large panel of Friedreich ataxia patient DNA samples

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Friedreich ataxia (FRDA) is a multi-system autosomal recessive inherited disorder primarily caused by homozygous GAA repeat expansion mutations within intron 1 of the frataxin (FXN) gene. The GAA repeat expansions may be pure (GAA)n in sequence or may be interrupted with regions of non-GAA sequence, such as (GAAGGA)n. To our knowledge there has been no largescale
study of FRDA patient DNA samples to determine the frequency of interruptions in GAA repeat expansions. Therefore, we have investigated a large panel of 258 FRDA patient and carrier DNA samples using GAA repeat PCR amplification and MboII restriction enzyme digestion, together with GAA repeat TP-PCR analysis. Our results demonstrate that the vast majority (87%) of FRDA GAA repeat expansions do not contain significant sequence changes that would result in abnormal MboII digestion profiles, indicating that they are primarily pure GAA repeats. However, a large number of samples (65%) do show small sequence variations at the 3’ end of the GAA repeat sequence as detected by TP-PCR. These results have specific implications in our understanding of FRDA disease progression and the more general understanding of trinucleotide repeat disease characteristics.

4. Frataxin deficiency leads to lipolysis alteration in skeletal muscle cells

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Type 2 diabetes (T2D) represents the major complication in Friedreich's ataxia (FRDA), however, the upstream events responsible for T2D remain to be determined. Skeletal muscle of FRDA patients show the same features of T2D including Impaired mitochondrial function, accumulation of intracellular lipids and insulin resistance. In this study we have analysed some of the possible mechanisms responsible for the increased risk of developing T2D in FRDA focusing our attention on the lipolytic system.

To generate a FRDA cellular model, we have used murine C2C12 myocytes silenced for frataxin (FXN). Also skeletal muscles obtained from a FRDA mouse model (KIKO) were analysed to corroborate the results obtained in vitro.

We have firstly analysed some of the main hallmarks of FRDA to validate the cellular model. We found that FXN mRNA expression and protein content was efficiently downregulated. FXN deficient cells displayed increased level of oxidatively damaged proteins as well as decreased mitochondrial activity. These events were also accompanied by up-regulated expression of atrogin and murf1, which are involved in the degeneration of myofibers. We found a significantaccumulation of intracellular lipids that was associated with altered level of key components of the lipolytic pathway. In particular, we detected a lowered activity of protein kinase A that resulted in the inhibition of hormone sensitive lipase (HSL). Moreover, we found decreased level of ABDH5 and adipose tryglyceride lipase (ATGL) that represent the enhancer and the rate-limiting enzyme of the lipolytic cascade respectively.

Our results indicate that impaired lipolysis in skeletal muscle could contribute to the development of T2D and suggest that therapeutic strategies aimed at targeting lipolytic enzymes could mitigate metabolic disturbances in FRDA.

5. Identification of specific brain metabolic dysfunctions in Friedreich's Ataxia using proteomic approach in an innovative Drosophila model

Jessica Ayache1 and Sophie Halliez1, Elodie Rousseau2, Laetitia Colomb3, Camille Garcia3, Herv. Tricoire4 and Jean-Michel Camadro1, V.ronique Monnier4 and Val.rie Serre1
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Friedreich's ataxia (FA) is the most common inherited recessive ataxia. In 98% of cases, FA is caused by an abnormal GAA trinucleotides repeat in the first intron of the frataxin gene. This mutation leads to a complex DNA structure formation, which sequesters the RNA polymerase, disabling frataxin expression at a basal level in cells. This is responsible for mitochondria decreased activity, lying at the root of severe metabolic disorders. This leads ultimately to cardiomyopathy, being the most frequent cause of premature death. There is an urgent need to better understand the molecular process involved in the pathology, to allow the development of effective therapeutic strategies.

Our study is based on the powerful Drosophila FA model. In fact, the frataxin Drosophila homologue fh shares a high degree of sequence homology with the human frataxin. Moreover, some phenotypes like cardiac dysfunction induced in Fh-depleted Drosophila can be rescued by the human frataxin expression, supporting conserved role of frataxin through evolution and between the two species.

By in vivo targeted RNA-interference methodology, we generated lineages that carry the constitutive transcriptional activator GAL4 through two drivers, used alone or combined and that trigger neurons or glia. We generated protein extracts from L3 larvae brains and analysed them by quantitative proteomic approach. We monitored proteins whose expression varies in these Drosophila FA models, allowing to look after specific biochemical pathways and precise biomarkers of the disease. This analysis could provide new clues and hypothesis about FA molecular mechanism, allowing to work on new genetic or pharmacologic strategies. We could test these new strategies on our Drosophila models first, before being validated in rodent models.

7. Defining the Frataxin G130V pathogenic mechanism in Friedreich's ataxia

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Introduction: Friedreich's ataxia (FRDA) is a neurodegenerative disease caused by reduced expression of the mitochondrial protein frataxin (FXN). Most FRDA patients are homozygous for large expansions of GAA repeats in intron 1 of the FXN gene, while a fraction of patients is heterozygous for a point mutation and GAA expansion). 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 and slower disease progression. We and others have demonstrated that the level of mature FXN protein is more prominently reduced in FRDA G130V samples compared to samples harboring homozygous expansions. Moreover, 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.
Methods: We employed mammalian expression systems along with RNA sequencing of FRDA G130V fibroblasts.
Results: FXN-G130V displays a punctate subcellular distribution and only partially co- localizes with FXN-WT, suggesting independent functions. We also confirmed that the reduced level of FXN protein observed in FRDA G130V fibroblasts occurs via a post- transcriptional mechanism, as steady-state FXN mRNA levels are comparable to those of unaffected carriers and nuclear export and splicing of FXN mRNA are unchanged.
Conclusions: No models or reagents exist to distinguish FXN-WT and FXN-G130V proteins in FRDA G130V patient specimens, and it remains unknown if FXN-G130V is processed and/or functional in disease-relevant tissues. We are using CRISPR/Cas9 to generate G130V cell line and mouse models to address these questions. Realization of this project will provide a mechanistic basis to specify the contribution of FXN-G130V isoforms to FRDA pathogenesis, necessary to develop therapeutic strategies tailored to FRDA G130V patients.

10. HAX-1 is a potential molecular biomarker for cardiomyopathies in Friedreich's Ataxia

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Frataxin deficiency, responsible for the disease Friedreich's Ataxia (FRDA), is crucial for cell survival since it critically affects survival of neurons, pancreatic beta cells and cardiomyocytes. The heart is frequently affected with typical manifestation of hypertrophic cardiomyopathy, which can progress to heart failure and death. Microarray analysis targeted at investigating FRDA pathogenesis revealed that frataxin overexpression correlates with overexpression of HS-1 associated protein X-1 (HAX-1), a family of proteins involved the protection of cardiomyocytes from apoptosis. Interestingly, HAX-1 heterozygous-deficient hearts exhibit increases in infarct size. Furthermore, HAX-1 coordinates network assembly of actin structures with KV3.3 channel, the dysfunction of which is correlated with Spinocerebellar ataxia 13 (SCA13). Frataxin and HAX-1 are therefore both involved in apoptosis regulation, a mechanism underlying the progression of cardiomyopathies.

Microarray analysis performed on lymphoblastoid cells derived from a FRDA patient stably reconstituted with wild type frataxin indicated HAX-1 as the highest up-regulated transcript ($FC=+2$, $p<0.05$). This result was further assessed at protein level by western blot analysis of I) HEK293 stably

transfected with empty vector compared to wild type frataxin, II) lymphoblasts and primary fibroblasts from FRDA patients compared to clinically unaffected heterozygous parents as well as healthy fibroblasts, and III) peripheral blood mononuclear cells of FRDA patients, heterozygous parents and non-correlated healthy controls. Moreover, qRT-PCR analysis revealed that frataxin and HAX-1 expression are correlated ($r= 0.95$, $p<0.05$) in a group of FRDA patients.

Our results suggest HAX-1 as an important gene possibly involved in the pathogenesis of a cardiac phenotype in FRDA. HAX-1 will be considered as a candidate for further evaluation as a potential biomarker for cardiomyopathies, potentially providing insight into their pathogenesis as well as prognostic information in these patients.

11. Molecular mechanisms underlying the atypical mild phenotype in Friedreich's ataxia patients with missense mutations

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Friedreich's ataxia (FRDA) is a recessive neurodegenerative disease caused by GAA trinucleotide repeats within intron 1 of the FXN gene, resulting in reduced levels of Frataxin (FXN) expression. 3-4% of FRDA patients are heterozygous carrying missense point mutations on one allele and GAA repeats on the other allele. Patients with G130V have significantly lower FXN levels than typical FRDA patients, yet present with an atypical mild phenotype.
While FXN is present in multiple forms including FXN1-210, FXN42-210, and FXN81-210, FXN81-210 has been the most studied. However, patients with G130V, have higher ration of FXN42-210 to FXN81-210 levels in both overexpression studies and patient fibroblasts, which may play a role in explaining milder phenotype. Cellular growth rate and phase contrast imaging was used to compare fibroblasts from control, typical FRDA, and G130V patients. Western blotting and confocal imaging was performed to compare FXN, mitochondrial ferritin, and aconitase levels. Cell iron content was quantified using a colorimetric assay, measuring absorbance at 593nm. Isotopologue analysis was used to label and follow Krebs cycle intermediates to study mitochondrial Krebs cycle flow. Finally, in-gel activity assays and NADPH absorbance at 340nm was used to measure cytosolic and mitochondrial aconitase activity.
Fibroblasts from patients with G130V, compared to typical FRDA patients, are healthier as measured by growth rate and cell morphology. They have increased mitochondrial ferritin and decreased total iron, suggesting absence of mitochondrial iron overload associated with typical FRDA, as well as increased mitochondrial Krebs Cycle flow. Furthermore, there is also increased cytosolic and mitochondrial aconitase activity, which is usually low in typical FRDA patients. Functional studies with FXN42-210-G130V are undergoing to investigate its functional capacity in iron-sulfur cluster biogenesis. Taken together, these findings provide insight into the pathogenic mechanisms associated with the atypical mild phenotype in FRDA patients with G130V missense mutation.

13. An in vitro study of the network connectivity in a Friedreich's Ataxia-neuronal model

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Introduction: Friedreich's Ataxia (FRDA) is an autosomal recessive ataxia caused by reduced expression of the mitochondrial protein frataxin. Although several mechanisms leading to neurodegeneration have been elucidated, alterations of neuronal activity and connectivity are still to be investigated. Neuronal function is highly sensitive to perturbed cellular homeostasis, including altered iron metabolism as found in frataxin-deficient cells.

Methods: We transfected hippocampal primary neurons, obtained from P2 rats, with validated siRNA against frataxin. We estimated frataxin expression (DIV) by western blot analysis. To evaluate neuronal excitability and network properties, cells were plated on Microelectrode Array (MEA) chips, where 60 electrodes allow the electrophysiological recordings of activity across the neuronal network, either under basal or treated conditions. Concomitantly, we investigated by fluorescence microscopy other neuronal parameters such as iron handling, ROS production, mitochondrial membrane potential, etc…

Results and Conclusion: In order to study how frataxin deficiency affects neuronal network activity, we established a neuronal model characterized by reduced level of frataxin and by the capability to form a mature and highly connected network. By RNA interference technology we induced a partial frataxin deficiency (about 50%) in primary neuronal cultures, which was maintained for 10-12 DIV. Validation of this model by analyzing the cellular parameters already characterized in human iPSC-derived neurons (Codazzi et al., 2016) is ongoing.

Preliminary MEA analyses revealed that control hippocampal neurons are highly active. Mild oxidative conditions (acute H2O2 treatment, 50 mM) resulted in increased burst activity and spike synchronization within the network. These experiments will be extended to siRNAtreated neurons and to iPSC-derived neuronal cells. This approach would offer a very sensitive in vitro assay to better understand neuronal functional alteration in FRDA and to evaluate the effects of therapeutic molecules.

14. CRISPR/Cas9 genome-wide screen to identify novel targets for the treatment of Friedreich's Ataxia

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Friedreich's Ataxia (FRDA) is the most common form of inherited ataxia and is caused by a GAA expansion in the intron 1 of the Frataxin gene. This expansion results in the depletion of the 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 find new molecular targets that can pave the way to the pharmacological upregulation of FXN.

Methods

Genome-wide knock-down or knock-out approaches have been extensively used to discover molecular pathways involved in physiological processes and disease. Here we propose a CRISPR/Cas9 genome-wide knock-out approach coupled with quantification of FXN expression to identify novel regulators of FXN expression.

Results

We have optimized an assay to detect with high sensitivity the endogenous levels of FXN in both healthy and patient cells. This assay can be exploited to identify cells that present an increase of FXN following a CRISPR/Cas9 genome-wide knock-out screen.

Conclusions

With this work we present a promising high throughput approach that aims to identify new regulators of FXN expression that could be exploited to develop a targeted therapy for FRDA.

15. Identification of p38 MAPK as a novel therapeutic target for Friedreich ataxia

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Introduction: Friedreich ataxia (FA) is an autosomal recessive neuro- and cardio- degenerative disorder caused by decreased expression of frataxin, a protein that localizes to mitochondria and is required for iron-sulfur-cluster (ISC) assembly.
Methods: We screened a random shRNA library and identified a synthetic shRNA (clone gFA11) that reverses the growth defect of FA cells in culture.
Results: Clone gFA11 decreases cytokine secretion in primary FA fibroblasts and reverts other changes associated with cell senescence. Using the Ingenuity software package, we found that the gene-expression profile induced by gFA11 is remarkably similar to the gene-expression profile induced by the p38 MAPK inhibitor SB203580. We found that p38 phosphorylation, indicating activation of the p38 pathway, is higher in FA cells than in normal control cells. Furthermore, siRNA knockdown of frataxin in normal fibroblasts also increases p38 phosphorylation. Treatment of FA cells with p38 inhibitors recapitulates the reversal of the slow-growth phenotype induced by clone gFA11.
Conclusions: These data highlight the involvement of the p38 MAPK pathway in the pathogenesis of FA and the potential use of p38 inhibitors as a treatment for FA.

18. Detecting known repeat expansions with standard protocol next generation sequencing

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Background- Repeat expansions cause over 20 neurogenetic disorders that can present with overlapping clinical phenotypes, making molecular diagnosis challenging. Ataxias are the most common of these including SCAs 1, 2, 3, 6, 7, 8, 10, 12, 17, 36, Friedreich ataxia (FRDA) and DRPLA. Single gene or small panel PCR-based methods are employed to identify the precise genetic cause, but can be slow and costly, and often yield no result. Genomic analysis via whole exome and whole genome sequencing (WES and WGS) is being increasingly performed to diagnose genetic disorders. However, until recently analysis protocols could not identify repeat expansions in these datasets.
Methods- A new method for the identification of repeat expansions using either WES or WGS was developed. Four retrospective cohorts of individuals with eight different known repeat expansion disorders, including SCA 1, 2, 6, 7 and FRDA, were analysed with the new method. Results were assessed by comparing to the known disease status. Performance was also compared to a recently published genotyping-based method, ExpansionHunter.
Results- Expansion repeats were successfully identified in WES and WGS datasets. The new method demonstrated very high predictive capabilities, achieving a median area under the curve (AUC) of 0.9. The new robust method achieved a median specificity and sensitivity of 0.99 and 0.75 respectively, compared to ExpansionHunter (median specificity = 0.99, median sensitivity = 0.56). These results were achieved regardless of whether the library preparation was PCR-free or not. Conclusions- The new method, called exSTRa (expanded STR algorithm), is available from https://github.com/bahlolab/exSTRa. It can be applied to existing WES or WGS data to identify likely repeat expansions. We demonstrate that exSTRa can be effectively utilised as a screening tool to interrogate WES and WGS sequencing data generated with PCRbased library preparations which can then be followed up with specific diagnostic tests.

23. Application of Next Generation Sequencing in a cohort of ataxic patients using a multi-gene panel approach

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Introduction: Hereditary ataxias (HA) are clinically and genetically heterogeneous conditions. Sanger sequencing in routine clinical investigation of HA patients has been replaced because the modern Next Generation Sequencing (NGS) approaches have proved to be more time- and cost- effective. Moreover, the clinical use of NGS has also broadened the etiologies in HA. With NGS applications, the diagnostic rate in HA is about 35% when using exome sequencing (ES), and about 20% when target resequencing panels (TRP) are applied. There are no studies examining na.ve patients with TRP strategies, however. In this study, we applied a TRP strategy in a cohort of ataxic patients as a first tier diagnostic approach.

Methods: We studied a consecutive group of 30 index cases with congenital, degenerative, or late onset ataxias. DNA sequencing adopted a TRP Haloplex platform, capturing 82 genes, and was performed on a MiSeq Illumina sequencer. All patients had been tested for pathological expansions in SCA1, 2, 3, 6, 7, 17 and for the intronic GAA expansion in FXN. Genotypes were analyzed using Ingenuity Variant Analysis. Probable and possible pathogenetic mutations were confirmed by traditional Sanger sequencing.

Results: We found pathogenetic mutations in 8 patients (27%), including 8 new variants (72%), detected in 5 different genes (PRKCG, SYNE1, PNPLA6, SPG7, SETX). SPG7, often mutated in hereditary spastic paraplegias (HSP), was the most common (38%) followed by PNPLA6 (25%).

Conclusions: In this study applying TRP to analyze na.ve HA patients we observed a diagnostic rate of 27%, a finding as yet less informative than ES which remains a method of choice in clinical diagnostic settings. Our study also confirmed that HA and HSP share several disease genes and could represent two extremes on a continuous spectrum.

26. Alteration of the growth cone dynamics in dorsal root ganglia neurons from the Friedreich ataxia YG8sR mouse

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Introduction: Appropriate levels of ATP and reactive oxygen species (ROS) generate a normal axonal growth, and together with the intracellular Ca2+ signaling, control the motility of growth cones and the axonal pathfinding. Based on the fact that FriedreichÅLs ataxia (FRDA) cells have altered mitochondrial ATP production, increased oxidative stress and calcium dysregulation, our hypothesis is that the deficit of frataxin affects the morphology and dynamics of the growth cones (GCs) in adult DRG neurons.
Methods: Morphometric and computer-based analysis of the GCs were performed in primary culture of DRG neurons from male Y47R and YG8sR mice at 2, 6 and 9 months of age and analyzed by Time-Lapse phase-contrast microscopy.
Results: The morphometric analysis from 2 month-old-mice exhibited a smaller area of GCs in YG8sR-DRG neurons than Y47R mice (91.24 vs. 69.69 µm2; **p=0.0013). In addition, twocustomized
computer-based analysis identified aberrant patterns in the dynamics of the GCs from YG8sR-DRG neurons. GCs from YG8sR-DRG neurons built shorter neurites (32.34 vs. 24.97 µm, *p=0.0491), at lower velocity (0.01 vs. 0.007
µm.sec-1, *p=0.0187), and with smaller axon turning angles (-68.14Åã vs. -42.97Åã, ***p=0.0006). The changes in morphology and the aberrant motion patterns present in the dynamics of the GCs of 2 month-old-mice were no longer evident for 6 and 9 month- old-mice.
Conclusions: The aberrant changes in the morphology and dynamics of the growth cones of adult DRG neurons from YG8sR mice suggest that the intrinsic ability to grow and to regenerate axons of DRG neurons would be altered as part of neuropathological process involved in FRDA pathophysiology.

31. Heart and nervous system pathology in compound heterozygous Friedreich ataxia

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Introduction: In a small percentage of Friedreich ataxia (FA) patients, the pathogenic mutation is heterozygous, consisting of a guanine-adenine-adenine (GAA) trinucleotide repeat expansion on one allele, and a deletion, point mutation, or insertion on the other. We report the heart and nervous system pathology of two compound heterozygous FA patients.
Methods: Patient 1, an 11-year-old boy (GAA, 825/c.11_12TCdel), had chorea, cardiomyopathy, and diabetes mellitus. He died from cardiorespiratory arrest. His heart weighed 288 g (expected normal: 122 g). Patient 2, a 28-year-old man (GAA, 707/exon 5 del), had cardiomyopathy and diabetes mellitus. He died from an intracerebral hemorrhage. The heart weighed 439 g (expected normal: 270-360 g).
Results: Microscopy of the left ventricular wall (LVW) of the heart showed cardiomyocyte hypertrophy, iron-positive inclusions, and disrupted intercalated discs. The cardiac lesions were similar to those in age-matched homozygous FA patients with cardiomyopathy and diabetes mellitus (boy, 10, GAA 1016/1016; woman, 25, GAA 800/1100). The neuropathology was also similar, including atrophy of the large neurons of the dentate nuclei (DN), hypoplasia of spinal cord and dorsal root ganglia, and loss of large axons in dorsal roots (DR). The DR in the 2 young FA patients and the adult FA heterozygote showed bizarre balloon-like expansions that reacted with antibodies to S100 and glial fibrillary acidic protein, indicating a central nervous system origin. Enzyme-linked immunosorbent assays of LVW and DN extracts of the 4 cases showed frataxin levels at or below the detection limits of the method (≤10 ng/g wet weight) (normal DN: 195Å}55 ng/g; normal LVW: 235Å}75 ng/g).
Conclusions: Disease manifestations in compound heterozygotes do not follow a uniform pattern but depend on the expression level of frataxin. Low tissue frataxin levels, rather than the specific type of mutation in the frataxin gene, also determine the pathological phenotype in FA (Supported by FARA).

32. Friedreich ataxia: developmental failure of the dorsal root entry zone

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Introduction: Dorsal root ganglia (DRG), dorsal roots (DR), and dorsal root entry zones (DREZ) of the spinal cord are vulnerable in Friedreich ataxia (FA). The depletion of myelinated fibers in the dorsal columns, more moderate loss of axons in the dorsal spinocerebellar tracts, and absence of nerve cells in the dorsal nuclei are fully explained by insufficient prenatal and perinatal centripetal growth of axons arising from DRG.
Methods: Segments of formalin-fixed upper lumbar spinal cord of 12 homozygous and 2 compound heterozygous FA patients were sectioned longitudinally to represent DREZ and stained for the glial fibrillary acidic protein (GFAP), S100, vimentin, the central nervous system (CNS)-specific myelin protein PLP, the peripheral nervous system (PNS) myelin proteins PMP-22 and P0, and the Schwann cell proteins alpha-dystroglycan, periaxin, and laminin. Additional methods were confocal microscopy and electron microscopy.
Results: Normal DREZ show a short dome-like extension of GFAP-, S100-, and vimentinreactive CNS tissue into DR and sharp demarcation of CNS and PNS myelin proteins. Alphadystroglycan, periaxin, and laminin form tight caps around these domes. In FA, CNS tissue
extends into DR over much longer distances, reaching up to 3 mm. The transition between CNS and PNS myelin is irregular. The ultrastructure of the CNS extensions into the DR shows heterogeneous fibrillary bundles.
Conclusions: These observations provide indirect evidence that frataxin deficiency in FA leads to incomplete demarcation between spinal CNS and PNS. During prenatal and perinatal development, neural-crest derived boundary cap cells provide guidance to DRG axons growing into the dorsal spinal cord and at the same time block the inappropriate intrusion of CNS glia into DR. It is likely that frataxin is required during a critical period of permissive (axons) and non- permissive (astroglia) border-control (Supported by Friedreich's Ataxia Research Alliance and New York State Department of Health).

33. Defining the effect of expanded GAA repeats on the kinetics of FXN transcription in Friedreich's Ataxia

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Introduction: Friedreich's ataxia (FRDA) is caused by reduced levels of the mitochondrial protein frataxin (FXN) as a result of large expansions of GAA trinucleotide repeats located in the first intron of the FXN gene. Transcriptional silencing of FXN is one of the primary targets for therapeutic intervention, therefore, understanding the exact molecular mechanism governing the GAA-induced silencing is essential for developing a precise therapeutic strategy. The expanded GAAs have been demonstrated to perturb both initiation and elongation of FXN transcription, however, high resolution analyses of the transcriptional kinetics in FRDA cells are lacking.

Methods: Analyses were conducted in FRDA and control iPSCs due to relatively high expression of FXN in these cells. Precision nuclear run-on sequencing (PRO-seq) assay was used to define nascent transcription with single nucleotide resolution. Inhibitors of transcription initiation and elongation were used to investigate the effect of the expanded GAAs. PRO-seq and RNA polymerase II ChIP-seq data were overlapped. Antibodies specific for total RNAPII, as well as Ser2 and Ser5 phosphorylated polymerase were utilized for immunoprecipitation.

Results: A comprehensive, high resolution transcription landscape of the FXN locus in FRDA and control iPSCs was determined. Strand-specific PRO-seq profiles demonstrated decreased transcription elongation in the FRDA cells. In addition, preliminary data indicate a decrease in recruitment of RNAPII machinery to the FXN promoter region accompanied by significant perturbations of the transition from initiation to productive elongation in FRDA cells. No significant antisense transcription initiation could be observed at the FXN locus in iPSCs.

Conclusion: Results of our transcription kinetics analyses in control and FRDA iPSCs indicate that interplay between transcription initiation-elongation of the FXN gene and especially the transition into processive elongation is affected in FRDA iPSCS. Therefore, simultaneous targeting of both initiation and elongation defects maybe required to achieve a therapeutically significant increase of FXN mRNA synthesis.

35. Synaptic pathology in in vitro and in vivo models of Friedreich's ataxia

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Proper distribution and supply of mitochondria are necessary for the normal maintenance of neuronal architecture and activity, including synaptic plasticity and function. Mitochondrial dysfunction in Friedreich's ataxia (FA) is likely to impact on some of these neuronal aspects. Although synaptic pathology in FA is largely unexplored, immunohistochemical studies in autopsy specimens from FA patients have provided evidence supporting the existence of synaptic abnormalities.

We aim to investigate synaptic abnormalities in cultured sensory neurons and in a mouse model of FA (KIKO mouse). We use molecular and cellular techniques, including fluorescence microscopy and electrophysiology recordings, and immunohistochemistry of spinal cords and muscles to study synaptic dysfunction in FA.

We have identified altered synapses and electrophysiological properties in KIKO sensory neurons, as compared to controls. Synaptic alterations are also present in the adult KIKO mouse, at both ends of the sensory neuron (that is, the spinal cords and the muscle spindles). We are currently testing the effects of therapeutic approaches aimed to correct Fxn-induced mitochondria dysfunction.

Our data suggests that synaptic dysfunction is an early, initiating event in FA pathogenesis.

40. NMR analysis of the direct complex between the FeS clusters IscU scaffold protein and frataxin

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Introduction: mutations of genes coding for proteins involved in the assembly of FeS clusters cause several human mitochondrial pathologies, including Freidreich's Ataxia (FRDA) [1]. The majority of FRDA patients are homozygous for an abnormal expansion of a GAA trinucleotide repeat in the first intron of the frataxin gene (FXN), leading to a severe reduction of protein expression levels [2]. A small but significant proportion of patients (i.e. 4%) are compound heterozygous for the GAA expansion on one FXN allele and for a mutation on the other. These patients present either the classical FRDA phenotype or an atypical, less severe clinical picture [3]. All clinically important mutations described in heterozygous FRDA patients affect highly conserved residues of frataxin [3]. On the other hand, the primary function of this protein is still under debate, and the specific contribution of its deficiency to the pathogenesis of both classical and atypical FRDA is unknown. One open issue is the involvement of frataxin in the FeS clusters assembly machinery. In this work, we explored by NMR the direct complex of wild type as well as several mutant frataxin proteins with IscU, the scaffold upon which the FeS clusters are assembled.

Methods: recombinant human (90-210) frataxin and IscU proteins were expressed in E. coli and purified to homogeneity by double-steps chromatographies. Their interaction was investigated by monitoring the changes in the sofast-HMQC spectra of 15N-labeled FXN upon addition of unlabeled IscU, either in the presence or in the absence of iron (as Fe3+ or Fe2+). The experiments were performed with wild type frataxin as well as with the pathological mutants G130V (associated to an atypical clinical presentation) and W155R (which results in the classical FRDA phenotype).

Results: we found that 1) wild type frataxin is able to directly interact with the scaffold IscU, as assessed by chemical shift perturbation of peaks associated to residues belonging to the Febinding region, and to a b-sheet portion of the protein; 2) this interaction is strictly dependent on iron (either Fe3+ or Fe2+); 3) several residues involved in the interaction are mutated in the heterozygous patients. Based on these results, we explored the complex of IscU with the two frataxin clinical mutant G130V and W155R. Both mutants, upon addition of IscU in the presence of iron, showed a chemical shift perturbation for residues in the Fe-binding region, as the wild type FXN. Anyway, subtle but significant differences, with respect of the wild type FXN, were observed in the b-sheet portion: only the G130V mutant, and not W155R, showed small shifts for peaks in this region, as the wild type FXN. Moreover, for G130V additional peaks were found to be influenced by the interaction, suggesting a larger plasticity of this mutant.

Conclusions: our data support the conclusion that the two investigated mutations of FXN have an influence of its interaction with IscU in vitro. Whether this finding could be related to their different clinical outcome requires further investigations, which are currently under way in our laboratories.

[1] Campuzano V. et al. (1997) Frataxin is reduced in Friedreich ataxia patients and is associated with mitochondrial membranes. Hum. Mol. Genet. 6, 1771-1780
[2] Coss.e M. et al. (1999) Friedreich's ataxia: point mutations and clinical presentation of compound heterozygotes.
Ann. Neurol. 45, 200-206
[3] De Castro M. et al. (2000) Genotype and phenotype analysis of Friedreich's ataxia compound heterozygous patients. Hum. Genet. 106, 86-92

41. Investigating the role of FAST-1 in Friedreich ataxia

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Friedreich ataxia (FRDA) is an autosomal recessive neurodegenerative disorder caused by a GAA trinucleotide repeat (TNR) expansion within the first intron of the FXN gene, leading to severe deficiency of FXN transcript. FRDA patients have disease-related epigenetic changes, which may be the underlying cause of FXN gene silencing. Furthermore, it has previously been shown in other TNR diseases that increased levels of antisense RNA expression can induce heterochromatin formation and epigenetic silencing of the corresponding sense gene.

The frataxin antisense transcript, FAST-1, is overexpressed in FRDA patient-derived fibroblasts, associated with depletion of CTCF, a chromatin insulator protein, and heterochromatin formation. We have overexpressed FAST-1 in both HEK293 and HeLa cell lines and we have identified a corresponding 30-70% decrease of FXN expression levels compared to control cells. Additionally, we identified a significant positive correlation between FAST-1 copy number and FAST-1 expression level ($R2 = 0.7$, $P = 0.04$) and a negative correlation between FAST-1 copy number and FXN expression level ($R2 = -0.58$, $P = 0.04$). Chromatin immunoprecipitation (ChIP) of FAST-1 overexpressing HeLa cells showed reduced occupancy of CTCF at the 5′UTR of the FXN gene. It is plausible that increased antisense transcription displaces CTCF from the 5′UTR and results in heterochromatin formation and FXN gene silencing.

To further investigate the role of FAST-1 in FXN gene silencing, we have used a small hairpin RNA (shRNA) to knock down FAST-1 in FRDA fibroblast cells. We found that knocking down FAST-1 increases FXN expression, but not to the level of control cells.

Since FAST-1 is associated with FXN gene silencing, inhibition of FAST-1 expression may be an effective approach for FRDA therapy.

42. Misregulation of microRNA expression in Friedreich's ataxia cells

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Introduction: Friedreich's ataxia (FRDA) is an inherited neurodegenerative disorder mainly characterized by muscle weakness, loss of coordination and heart muscle abnormalities. FRDA is caused by expansion of GAA nucleotide repeats in the first intron of the Frataxin gene (FXN), which leads to decreased protein production. Frataxin is involved in biogenesis of iron-sulfur clusters and heme, thus its diminished levels disrupt iron and heme homeostasis. Interestingly, imbalanced heme and iron levels affect maturation of microRNAs (miRNAs), small non-coding RNAs that control target gene expression. Recently, misregulation of miRNA processing has been observed in neurodegenerative diseases indicating miRNA involvement in the pathogenesis of these disorders. Since miRNA levels are changed in FRDA, we hypothesize that reduced frataxin levels disturb expression and processing of miRNAs through impaired iron/heme metabolism. Methods and results: To verify this hypothesis, miRNA sequencing was conducted on 15 fibroblast lines from FRDA patients and 15 unaffected control lines. Based on the outcome, a set of 10 aberrantly expressed miRNAs in FRDA patients was selected. Further RT-PCR analysis narrowed down this set to two significantly changed miRNAs in FRDA fibroblasts, namely 10a-5p and 148a-3p, which showed over two-fold increase in expression compared to unaffected controls. Next, luciferase assays were used to evaluate how these selected miRNAs affect the expression of their target genes. Among analyzed candidate genes were those associated with heme and iron metabolism as well as those involved in neurodegeneration. To further establish the miRNA signature in FRDA, fibroblasts from several patients and controls were reprogrammed to induced pluripotent stem cells. Neurons and cardiomyocytes will be differentiated from these cells for comprehensive studies to establish the interplay between miRNA and heme/iron metabolism at the molecular level. Conclusions: These results reveal aberrant miRNA processing in FRDA cells, indicating a new therapeutic target for FRDA treatment.

48. mTOR is differentially activated in in vitro and in vivo models of Friedreich ataxia.

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Introduction

To find pathogenic pathways that may represent therapeutic targets for Friedreich ataxia (FRDA), we investigated the possible involvement of the mechanistic target of rapamycin (mTOR). A systematic study of this pathway in FRDA has never been performed, so it is unknown if and how frataxin (Fxn) and mTOR are connected. mTOR is involved in key metabolic processes known to be affected in FRDA, such as energy metabolism and iron homeostasis.

Methods

mTOR activation was assessed by Western blotting by quantifying phosphorylation of its substrates (S6K, S6, 4EBP1) in in vitro models, such as Fxn knock-down SH-SY5Y and 293T cells and patient iPS-derived neurons; and in vivo models, such as KIKO mice and liver and heart Fxn conditional KO mice. The expression level of genes positively regulated by mTOR, such as mevalonate kinase (MVK) and glucose-6-phosphate dehydrogenase (G6PD) was also measured by qPCR.

Results

In cell models with reduced Fxn expression, mTOR activation appeared to be overall downregulated.

However, in in vivo models mTor activation was tissue-specific. In conditional KO mice, mTor activation was increased in liver and heart. In KIKO mice, mTor activation was increased in the cerebellum, while it was decreased in skeletal muscle. Significantly reduced MVK expression was observed only in patient IPS-derived neurons.

Conclusions

Our preliminary data suggest that in in vitro models, mTOR activation is down-regulated when Fxn expression is decreased. Conversely, in in vivo models of FRDA its regulation is highly tissue-specific. In vivo, different tissue-specific compensatory mechanisms absent in the cell culture models, including homeostatic responses involved in iron metabolism, could be responsible for differential mTOR activation.

51. Frataxin-deficient cardiomyocytes present an altered thiol-redox state

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Introduction: Friedreich ataxia is a neurodegenerative disease accompanied by hypertrophic cardiomyopathy. This disease is caused by deficient expression of frataxin, a mitochondrial protein that has been related to iron homeostasis, energy metabolism, and oxidative stress. We have recently set up a cardiac cellular model of Friedreich Ataxia based on neonatal rat ventricular myocytes (NRVMs) and lentivirus-mediated frataxin RNA interference. As these frataxin-deficient NRVMs present signs of oxidative stress, we decided to explore the presence of protein thiol modifications in this model.

Methods: The presence of reversible oxidized cysteine residues was investigated using the thiol-reactive fluorescent probe Bodipy-iodoacetamide and 2D-gel electrophoresis. The presence of glutathionylated proteins was analyzed using antibodies against glutathione. Modified proteins were identified by mass spectrometry.

Results: We identified three proteins with altered redox status in frataxin-deficient NRVMs: Electron transfer flavoprotein-ubiquinone oxidoreductase (ETFD), Dihydrolipoyl dehydrogenase (DLDH) and ATP synthase subunit alpha (ATPA). As DLDH is involved in lipoic acid turnover, we investigated the status of this cofactor in more detail. We found that total protein-bound lipoic acid levels are not affected in frataxin-deficient NRVMs, but their redox status is compromised as it is found in a more oxidized form. We also analyzed the presence of glutathionylated proteins and we found that actin was glutationylated in frataxin-deficient NRVMs.

Conclusions: These results are indicative of an altered thiol-redox state in frataxin- deficient NRVMs that could contribute to the cardiac pathology. Therefore, we are currently exploring the potential protective effect of thiol-containing antioxidants on this model.

52. Early dysregulated cardiac mitochondrial biogenesis and OXPHOS system in the KIKO mouse model of Friedreich Ataxia

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Introduction

Friedreich Ataxia (FRDA), an autosomal recessive neurodegenerative disease, is the most common form of inherited ataxia resulting from deficiency of frataxin, a highly conserved mitochondrial protein crucial for Fe/S cluster formation and ATP production. The mean age at death is 38 years old due to coexistence of cardiomyopathy. Frataxin deficiency is associated with mitochondrial dysfunction in FRDA patients and models. However, early mitochondrial pathophysiology in FRDA heart remains elusive.

Methods

Using the frataxin knock-in/knockout (KIKO) mice of FRDA, we sought to investigate if mitochondrial biogenesis and OXPHOS complex are altered in KIKO heart at presymptomatic age by examining the levels of a master mitochondrial biogenesis regulator, PGC-1a, and OXPHOS complex markers using Western blotting and OXPHOS complex enzyme activity microplate assay kits.

Results

At presymptomatic age, levels of PGC-1a are increased by 51% in heart homogenates of KIKO mice compared with age-matched controls, while the frataxin levels are reduced by 90%, suggesting early dysregulation of mitochondrial biogenesis in response to frataxin-deficiencyinduced
mitochondrial stress. The protein levels of OXPHOS Complex III, IV and V markers UQCRC2, MTCO1 and ATP5A, are decreased by 20%-33 % respectively, whereas levels of Complex I core subunit NDUFB8 are increased by 44% and Complex II subunit SDHB remains unaltered. Furthermore, enzyme activities of Complex II and IV are comprised, whereas Complex I activity remains unaltered, suggesting dysregulated cardiac mitochondrial OXPHOS systems in presymptomatic KIKO mice.

Conclusions

Our findings identify early dysregulation of cardiac mitochondrial biogenesis and the OXPHOS system as a potential mediator of cardiomyopathy, thereby providing a potential therapeutic target for in FRDA patients.

55. A CRISPR approach for investigating epigenetic silencing in Friedreich ataxia

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Introduction: Friedreich ataxia (FRDA) is caused by an expanded GAA repeat in intron 1 of the FXN gene that induces heterochromatin formation and silencing of the promoter. The FXN promoter is unmethylated in both FRDA and non-FRDA cells.

However, the CpG island shore (CGI shore), an area adjacent to the promoter, becomes hypermethylated in FRDA. Because hypermethylation of CGI shores is known to negatively regulate gene expression, we hypothesized that FXN CGI shore methylation contributes to epigenetic silencing in FRDA.

Methods: shRNA-mediated knockdown of DNMT3A and DNMT3B was carried out in patientderived
lymphoblastoid cells. qRT-PCR was used to measure FXN transcript levels. Methylationspecific qPCR (MS-qPCR) was used to measure DNA methylation. Furthermore, to modify DNA methylation specifically at the FXN locus, we designed a CRISPR-Cas9 strategy to target DNMT3A to the FXN CGI shore via nine different guide RNAs. HEK293T cells were transfected with these and deactivated Cas9 tethered to DNMT3A. DNA methylation was measured at CpG sites within the CGI shore with MS- qPCR, methylation-sensitive high-resolution melting (MSHRM),
and bisulfite sequencing.

Results: Knockdown of DNMT3A and DNMT3B reduced DNA methylation at the FXN CGI shore, and increased FXN transcript levels. Targeting dCas9-DNMT3A to the FXN CGI shore significantly increased DNA methylation at the FXN locus.

Conclusions: DNMT3A and DNMT3B facilitate DNA hypermethylation of the FXN CGI shore, which contributes to silencing of the FXN gene in FRDA. CRISPR-Cas9 is a powerful tool to investigate the molecular mechanism of epigenetic silencing in FRDA.

57. Mitochondrial calcium transporter NCLX, the NFAT3 transcription factor and mitochondrial permeability transition pore become altered in cell models of Friedreich ataxia.

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Introduction: Previous results from our group showed that decreased frataxin levels in DRG neurons provoked an alteration of calcium homeostasis, neurite degeneration and apoptotic cell death (1). In cardiac myocytes, frataxin deficiency lead to mitochondrial swelling (2) suggesting the opening of mitochondrial permeability transition pore (MPTP). Therefore, we have analysed the downstream effects of frataxin deficiency by studying the relationship between MPTP, mitochondrial calcium transporters (MCU and NCLX) and NFAT, a transcription factor that, upon dephosphorylation by calcineurin (a calcium-dependent phosphatase) becomes active, travels to nucleus and promotes cardiac hypertrophy (3) and neurodegeneration of dopaminergic neurons (4).

Methods: We used primary cultures of cardiac myocytes and dorsal root ganglia (DRG) neurons from newborn rats. Reduction of around 80% of frataxin levels in these cells is achieved by transduction with lentivirus containing shRNA silencing sequences. MPTP opening was checked by Cobalt-CalceinAM quenching method.

Results: After frataxin depletion, we observed that, in cardiac myocytes, calcein fluorescence inside mitochondria was quenched by CoCl2 treatment indicative of the MPTP opening. These cells also displayed reduced levels of NCLX, a mitochondrial transporter for calcium efflux. Interestingly, DRG neurons and lymphoblastoid cell lines obtained from Friedreich Ataxia patients also display reduced levels of the transporter. We also show that, after frataxin depletion, NFAT becomes dephosphorylated in both cardiomyocytes and neurons. Finally, cyclosporin A, a drug that closes MPTP, is able to reverse the mitochondrial disarrangements observed in cardiac myocytes, promotes NFAT phosphorylation and restores cell viability in DRG neurons.

Conclusion: The connection between decreased NCLX levels, induction of MPTP opening and NFAT dephosphorylation provides new molecular clues to understand the deleterious downstream effects of frataxin deficiency. These results open a possibility for repurposing cyclosporin A or compounds targeting MPTP as an approach to FA treatment.

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1. Mincheva-Tasheva S. et al. 2014. Hum Mol Genet. 23:1829-41
2. Obis E. et al. 2014. Free Radic Biol Med. 73:21-33
3. Houser SR, Molkentin JD. 2008. Does contractile Ca2+ control calcineurin-NFAT signaling and pathological hypertrophy in cardiac myocytes?. Sci Signal. 1(25):pe31.
4. Luo J. et al. 2014. A calcineurin- and NFAT-dependent pathway is involved in α-synuclein-induced degeneration of midbrain dopaminergic neurons. Hum Mol Genet. 23:6567-6574

64. Nitric Oxide prevents Aft1 activation and metabolic remodeling in frataxindeficient yeast

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Introduction: In yeast, frataxin deficiency activates Aft1, a transcription factor which induces the expression of proteins involved in iron uptake. The mechanisms causing this activation are not completely understood. It is assumed that loss of iron-sulfur biogenesis may prevent an iron-sulfur dependent signal to retain Aft1 in the cytosol. However, previous research from our group indicates that activation of Aft1 occurs in the absence of iron-sulfur deficiency. Besides Aft1 activation, frataxin deficiency also leads to metabolic remodeling and to induction of Yhb1, a nitric oxide (NO) detoxifying enzyme (Moreno-Cerme.o, Alsina et al., BBA 2013;183:3326). In this work, we investigate the relationship between NO and Aft1 activation in frataxin- deficient yeasts.

Methods: we have used conditional (tet-regulated) frataxin and Grx5 mutant yeast strains. Increased NO levels have been achieved by null Yhb1 mutations and by exogenous exposure to sodium nitroprusside, an NO donor. Metabolic remodeling has been evaluated by targeted proteomics. Iron quantitation, western blot, qPCR and microscopy have been used to evaluate Aft1 activation.

Results: we have observed that increased NO levels prevent Aft1 activation in frataxindeficient yeasts. This phenomenon is not observed when Aft1 is activated by iron scarcity, oxidative stress or impaired iron-sulfur biogenesis (Grx5 deficiency). In addition, NO also prevents the metabolic remodeling caused by frataxin deficiency.

Conclusion: a major conclusion of this work is that the mechanism that leads to Aft1 activation in frataxin-deficient yeasts must differ from the one promoted by iron-sulfur deficiency or iron scarcity. Our hypothesis is that frataxin deficiency leads to the presence of anomalous iron species in mitochondria that can compromise iron bioavailability and activate a signaling cascade that results in Aft1 activation. NO would chelate these iron species and form ironnitrosyl complexes which would increase iron bioavailability and avoid Aft1 activation.

Acknowledgments: This project has been funded by SAF-2013-44820-R from Ministerio de Economia y Competitividad (Spain)

65. Deciphering metabolic dysfunctions in Friedreich's ataxia using quantitative proteomic approach

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Friedreich's ataxia (FRDA) is caused by mutations in the FXN gene encoding frataxin protein arising from an unstable hyperexpansion of GAA triplet repeat in the first intron of the gene. This hyperexpansion leads to FXN gene silencing by epigenetic modifications. Rarely, gene defects found in FRDA patients are loss-of-function mutations. However despite many efforts to overcome any of these abnormalities, there is currently no efficient treatment to cure or even stop the progression of this disease, mostly because many aspects of the pathological consequences of frataxin depletion are still not fully understood. The precise role of frataxin is still under debate. A key function of frataxin in Fe- S cluster biogenesis has now been clearly pointed out, but how its role in this essential cellular pathway correlates with the pathophysiology of FRDA needs to be further investigated.

No systematic quantitative proteomic studies have been reported so far concerning this disease. The use of this metabolomic approach offer an opportunity to screen and analyze several biochemical pathways at once, and provide a dynamic and a functional integration of metabolism.

To precise the consequences of frataxin depletion, we used a quantitative proteome analysis on heterozygous FRDA lymphoma B-cells (650 and 1300 GAA repeats) compared with controls as a method to study steady-state and perturbation induced-changes in protein profiles. Approximately 200 proteins showed statistically significant fold changes between FRDA and control cells. The differences in selected proteins were verified by Western blotting or enzymatic assays. Differentially abundant proteins were enriched or decreased in cellular pathways previously implicated in FRDA including mitochondrial respiratory chain, oxidative stress and iron homeostasis. Interestingly, new metabolic pathways have been highlighted in our study which could be future targets for novel therapeutic strategies in FRDA, which still lacks a cure.

67. Dissection of epigenetic mechanisms underlying the GAA-mediated FXN silencing in Friedreich's ataxia to identify FXN up-regulating compounds

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Several human diseases, including neurogenerative disorders, are associated with reduced gene expression. In Friedreich's ataxia (FRDA), the molecular mechanisms of pathology are associated with epigenetic silencing of the frataxin gene (FXN). It has been shown that expanded GAA repeats induce a repressive heterochromatin environment at the FXN locus. Silent heterochromatin is characterized by the presence of histone modifications, such as H3K9 methylation and the absence of acetylated histones. Therefore, the epigenetic changes reported at the FXN locus in FRDA, including increased levels of methylated histones H3K9me2 and H3K9me3 and reduced acetylation of histones H3 and H4, clearly demonstrate epigenetic repression of the FXN gene in FRDA.

Our group has been working on the silencing mechanisms of FXN and is particularly interested in the epigenetics driving the pathology. Histone deacetylase 3 (HDAC3) and euchromatic histone-lysine N-methyltransferase 2 (EHMT2/ G9a) have been associated with epigenetic changes of the FXN locus. We are directly targeting these proteins in a human GAA repeat expansion reporter model of FRDA using siRNA knock down to assess FXN expression. Additionally, we are screening an epigenetic probe library containing compounds which target several molecules involved in methylation and demethylation of histones and other proteins, as well as heterochromatin formation and remodelling. Identification of compounds that restore FXN expression will help us to characterize the molecular basis of FXN silencing and will contribute to finding novel therapeutic approaches for this fatal disease.

TRANSLATIONAL MODELS OF DISEASE

76. Characterization of iPS-derived Friedreich ataxia cardiomyocytes

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Introduction: Hypertrophic cardiomyopathy, leading to arrythmias and/or heart failure, is the leading cause of premature death in Friedreich ataxia (FA) patients. Several groups have successfully derived human cardiomyocytes from FA iPS cells and reported morphological differences compared to normal controls. However, few functional studies have been reported. We derived cardiomyocytes from FA iPS cells in order to 1) study the molecular mechanisms that underlie the pathophysiology of FA cardiomyopathy, and 2) identify phenotypes that might be exploited for drug testing.
Methods: iPS cells derived from two FA patients and two control subjects were differentiated into cardiomyocytes. We measured calcium transient concentrations and molecular markers of cardiac hypertrophy by RT-PCR.
Results: We confirmed the successful differentiation of iPS cells into cardiomyocytes. Calcium concentrations in the FA cardiomyocytes were consistently higher than in the controls. We also found increased expression levels of GATA-4 and Natriuretic Peptide A, both markers of cardiac hypertrophy. PGC1-alpha was also overexpressed in the FA cardiomyocytes compared to controls.
Conclusions: Our study identified increased calcium concentrations, and increased expression of genes associated with cardiac hypertrophy, as markers of iPS-derived FA cardiomyocytes. Recently, Crombie et al. (Aging 9:1440, 2017) derived cardiomyocytes from FA iPS cells and found 1) an increase in heart rate variability, and 2) a decrease in intracellular calcium concentrations, which was paradoxically rescued by nifedipine, a calcium channel blocker. The discrepancies between our results and those of Crombie et al. suggest that subtle technical variables may contribute to specific phenotypes in iPS- derived FA cardiomyocytes in culture and that caution should be exercised presently in interpreting results from these cells.

77. Understanding Friedreich’s ataxia neuropathophysiology using a new conditional neuronal mouse model.

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Friedreich's ataxia (FA), the most common recessive ataxia, is characterized by sensory and spinocerebellar ataxia and hypertrophic cardiomyopathy. Proprioceptive neurons within the dorsal root ganglia (DRG) are one of the primary affected cells in FA patients. FA is caused by reduced levels of frataxin (FXN), an essential mitochondrial protein involved in the biosynthesis of iron-sulfur (Fe-S) clusters. FXN depletion leads to a Fe-S cluster protein deficit, mitochondrial dysfunction, iron dysregulation and cellular dysfunction. The molecular mechanism underlying neuronal degeneration has not been well established. To decipher the pathological mechanisms in proprioceptive neurons, a new conditional neuronal mouse model (cKO), based on the Cre/LoxP technology, was generated, using the Cre Recombinase expressed under the parvalbumin promoter. Parvlb cKO mice present a FXN depletion in DRG proprioceptive neurons, starting at E17.5, in Purkinje cells of the cerebellum at p40 and in interneurons of the brain at 21.5 weeks. Interestingly, we showed that proprioceptive neurons, which represent only 7.5% of the DRG cell population, express between 50 and 70% of the total FXN of the DRG. Moreover, lumbar DRG express more FXN than cervical DRG. Parvlb cKO mice develop a severe and progressive ataxic phenotype assessed by different behavioural tests and a specific decrease of the sensory wave, revealed by electrophysiological studies. At the molecular level, we identified a deficit of a Fe-S protein, the Succinate Dehydrogenase, in proprioceptive neurons and in Purkinje cells, followed by cellular iron dysregulation, in agreement with elements observed in non-neuronal mouse models. To decipher the downstream events following FXN depletion, RNAseq analysis of DRG was performed and an upregulation of genes known to be expressed by sensory neurons following axonal damage (Regeneration Associated Genes) was identified. Further molecular analyses are ongoing to elucidate the mitochondrial and cellular defects in neurons. Understanding FA neuropathophysiology is critical to develop therapeutical strategies and to identify biomarkers that are essential to validate therapeutical approaches such as gene therapy.

78. A human iPSC-based cardiac model of Friedreich's Ataxia for drug discovery and patient stratification using all-optical electrophysiology

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Introduction:
Nearly two thirds of all Friedreich's Ataxia (FA) patients present with cardiac impairment or thickening of the myocardium and heart disease is reported as the most common cause of death in FA. Cardiac impairment has been evaluated through traditional analysis of cardiac function in both FA patients and mouse models. Such phenotypes, however, have not yet been recapitulated in in vitro cellular models, limiting the mechanistic understanding of FA and the ability to evaluate new therapeutic approaches.
Methods:
Using a proprietary all-optical electrophysiology platform (Optopatch) that enables simultaneous readout of action potentials (APs) and calcium transients (CTs) under paced conditions, we compare the electrophysiological phenotype in cardiomyocytes (CMs) expressing different levels of frataxin protein. To further elucidate the mechanism of cardiac impairment, these cells are treated with chemical stimuli (iron, beta-adrenergic, ryanodine receptor-targeted, etc.) to reveal the possible cause of phenotypic differences between FA and control samples.
Results:
We have completed preliminary studies with CMs derived from FA-patient and zinc fingercorrected iPSCs using Optopatch. CMs successfully paced at 1.5 Hz and voltage and calcium waveforms were measured with high fidelity. In addition, we have also made preliminary comparisons between the disease and corrected cell lines. We see an increase in rise time, decrease in AP80 duration, and a decrease in Ca2+ amplitude of the disease compared to corrected CMs. Work is underway to confirm the correlation between these measurements and FXN expression levels and to identify potential mechanisms of cardiac impairment in FA CMs.
Conclusions:
Our preliminary data shows the feasibility of using this approach for phenotypic evaluation of electrophysiological changes from FA-patient derived CMs. Broadly, this approach will support the functional validation of increased FXN expression in cardiomyocytes, screening of therapeutics, and open the opportunity for use in patient stratification.

80. Epigenetic editing of the Frataxin (FXN) locus by re-purposing CRISPR/CAS9 – targeted epigenetic editing with heterochromatin antagonists specifically reactivates the FXN gene in living cells?

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Background:
Aberrant epigenetic silencing is a fundamental disease mechanism in FA. We reasoned that its dynamic nature might permit targeted therapies to reactivate frataxin (FXN) expression through epigenetic editing, which may be more feasible than trinucleotide repeat excision within disease specific tissues. Targeted epigenetic modification of the frataxin locus, through removal of histone methylation (H3K9me3 and H3K27me3), DNA methylation and addition of histone acetylation are a powerful means to identify epigenetic regulators important in gene silencing, an d promote frataxin expression without the unwanted secondary effects found to occur with other investigative and therapeutic approaches (e.g. gene knockout and histone deacetylase inhibitors). Recent studies have identified naturally occurring H3.3 mutations as powerful inhibitors of both H3K9me3 and H3K27me3 histone modifications (1-3). This combination of both constitutive and facultative heterochromatin modifications at a single locus is highly unusual and occurs at the FXN gene in FA patients. Recently, such H3.3 mutant peptides have been shown to be powerful inhibitors of H3K9me3 and H3K27me3 and suppressors of heterochromatin-mediated position effect variegation in Drosophila in vivo.
Given that the FXN gene is decorated with the unusual combination of both H3K9me3 and H3K27me3 we reasoned that targeting of these mutant H3.3 peptides directly to the FXN locus might be an effective way of overcoming pathological heterochromatin-mediated silencing.
Methods:
Nuclease dead Cas9 protein fused to several epigenetic modifiers including the histone acetyl transferase, p300, mutant H3.3 peptides and transcriptional activators (VPR) were targeted to either the FXN promoter, upstream or downstream of the GAA expansion within the frataxin locus in FA cell lines. Frataxin expression was assessed by qRT-PCR. Off-target effects and frataxin transcriptional network analysis will be assessed through RNA-Seq and ChIP- Seq. Validated constructs will then be optimised for use with AAV9 for CNS targeting and delivered to FA transgenic mice.
Results:
Up to a 2-fold increase in Frataxin expression occurred when the frataxin locus was specifically targeted with a transcriptional activator, the histone acetyltransferase, p300 and more recently the novel histone H3.3 peptide mutants, which have been shown to inhibit both H3K9me3 and H3K27me3. Significant upregulation was noted when H3.3 was targeted downstream of the GAA and H3K9M was targeted to the promoter (two tailed t test, $p<0.05$). Significant downregulation was noted upon targeting H3K27M to the promoter and downstream of the of the GAA repeat (two tailed t-test, $p<0.05$). We have, therefore, identified both specific DNA elements within the Frataxin locus within the promoter region and flanking the GAA_repeat itself as well as novel heterochromatin inhibitors using mutant histone-peptide mimics capable of upregulating FXN expression.
Conclusions:
Epigenetic editing of the frataxin gene and targeted transcriptional activation using dCas9 fusion proteins are a novel and powerful methodology to dissect the epigenetic requirements for FXN silencing and its reversal in FA.
References:
Herz, H.-M. et al. "Histone H3 Lysine-To-Methionine Mutants As A Paradigm To Study Chromatin Signaling". Science 345.6200 (2014): 1065- 1070.

2. Lewis, P. W. et al. "Inhibition Of PRC2 Activity By A Gain-Of-Function H3 Mutation Found In Pediatric Glioblastoma". Science 340.6134 (2013): 857-861.
3. Chan, K.-M. et al. "The Histone H3.3K27M Mutation In Pediatric Glioma Reprograms H3K27 Methylation And Gene Expression". Genes & Development 27.9 (2013): 985-990.

82. Application of nanotechnology in FRDA drug research.

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Present age is the age of interdisciplinary collaborative research. The collaboration of different disciplines has advanced research at very fast rate. Nanotechnology is concerned with the synthesis of nano sized material particles, study of their properties and their applications. Nanoparticles have novel optical, electronic, magnetic properties that are not found in individual molecule or bulk solid. The living organisms are made up of cells and proteins which are of nano size. Therefore, nanotechnology finds promising application in medical science. Nanoparticles can be made body compatible by coating them with a suitable surfactant. In recent years nanoparticles have found application in cancer therapy, drug targeting and drug delivery. A targeted drug delivery system (TDDS) releases the drug at a specific bio- site in a controlled way. TDDS can prevent drug degradation and eliminate biological barriers. It has now been possible to prepare magnetic nanoparticles sensitive to specific pH value. The magnetic naoparticles can control the speed of drug delivery at the destination and it is possible to skip the regions quickly where the drug has adverse effect. For FA there is no cure. Drugs for FA are under trial stage but there is no evidence of nanotherapeutic drug delivery system (NDDS). The use of NDDS shall be effective for FRDA drug trial and it is very essential when the drugs are administered for long period. Joseph F. Nabhan and team made an attempt in which nanoparticles were used to introduce frataxin to maintain frataxin leval. Recently the authors of this paper presented a paper on “Magnetic nanoparticles approach to control degeneration in FA” in 14th international conference of Nanotechnology and Nanomaterials. However this theoretically justified approach needs experimental verification.

83. A Drosophila cell-based assay for high-throughput screening of genetic modifiers of FXN transcriptional silencing mediated by the GAA repeat expansion.

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Introduction: The GAA repeats expansion form unusual DNA structures affecting gene expression. These structures include triplexes and a related structure known as sticky DNA that could affect transcription by sequestering transcription factors or RNA polymerase or leading the formation of an RNA:DNA hybrid. It has been also shown that the region flanking the GAA repeats in the FXN gene is enriched for epigenetic marks characteristic of transcriptionally repressed regions of the genome. The FRDA repeats might trigger the formation of heterochromatin that could spread to adjacent sequences.
Methods: To identify potential genetic factors involved on the FXN transcriptional represion mediated by the GAA repeat expansion, we obtained a Drosophila cell model containing the FXN intron 1 with a pathological number of the GAA repeats. In the model cells, the expression of a reporter gene (firefly luciferase) is under the effect of an expansion of ≈300 GAA repeats. The experimental design is based on the use of the S2R+ Drosophila cells and the pACMAN methodology allowing the generation of two stable cell lines, the model line and the control line carrying 300 GAA and 9 GAA repeats respectively. Both lines have a pACMAN-renilla luciferase construct to normalize the results. The expression of luciferases was controlled by the UAS sequences and the system is analyzed by means of the GAL4-UAS methodology, using an inducible GAL4 driver by copper (pMT-GAL4).
Results: Luciferase activity was measured as a ratio of luminescence between firefly and renilla luciferases. This activity was 2.5 lower for the 300 GAA cell lines than the control line with 9 GAA repeats at 1mM Cu. Testing the system, we co-transfected the model cell line with a diap1 dsRNA observing a reduction of 5 times on firefly luciferase activity because diap1 is involved in apoptosis control and its depletion kills the cells, indicating that the RNAi machinery works in this model. We found that the effect of knocking down several genes involved in the heterochromatin formation increase significantly the firefly luciferase expression.
Conclusions: We obtained a cell based-assay useful for high-throughput screening of a large collection of dsRNAs to identify genetic factors involved in the repression mechanisms of FXN in FRDA. We expect to identify Drosophila genes which human homologous could change the epigenetic marks associated with the GAA-mediated heterochromatinization.

84. Mouse models of Friedreich's Ataxia

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Introduction: Friedreich's Ataxia (FRDA) is an autosomal recessive ataxia caused by a mutation in the frataxin gene. This mutation is characterized by an expanded tri-nucleotide (GAA) repeat within the first intron of the gene. This expansion leads to reduced expression of frataxin, a ubiquitously expressed protein that acts in iron sulfur cluster and heme biosynthesis. Insufficiency in frataxin causes decreased activity of iron-sulfur cluster enzymes such as aconitase and the mitochondrial respiratory chain complexes. The approach to model FRDA in the laboratory mouse entails knocking out endogenous Fxn expression and replacing it with mutant FXN containing large GAA repeats either through transgenesis or a targeted approach.
Methods: The Jackson Lab currently has over 15 different mouse models of FRDA under development or for distribution from the scientific community. These models have been genetically standardized and rederived into high barrier facilities for the scientific community. The repository at The Jackson Lab has performed a comprehensive phenotyping program cross comparing these models. Current publicly available mouse models for FRDA fall short at recapitulating many of the pathological and physiological features of the disease in humans.
Results: The newly available genome editing technologies afford us the opportunity to optimize the current FRDA collection and make new models for FRDA at an efficiency never before seen. These models include new BAC transgenic models that carry human genes expressing low levels of human Frataxin, conditional alleles that do not require licenses, knock–in models with larger repeats, and siRNA approaches to generating relevant alleles.
Conclusion: Our aim at the Rare and Orphan Disease Center at JAX is to genetically standardize the disease collection, delineate phenotypic parameters for each of the available FRDA models, and work to provide better models to the FRDA community to aid in the advancement of therapeutic discovery.

85. Histological characterization and drug screening on a Drosophila cardiac model of Friedreich Ataxia

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Friedreich Ataxia {FA) is characterised by progressive degeneration of the central and peripheral nervous system, hypertrophic cardiomyopathy and increased incidence of diabetes. FA is caused by reduced levels of frataxin, a highly conserved mitochondrial protein. Drosophila appears as an adequate animal model to study pathogenic mechanisms involved in FA and to evaluate therapeutic interventions. We have previously developed a Drosophila cardiac model of FA, in which the fly frataxin is inactivated specifically in the heart by RNAi interference using a RU486-inducible system. These flies exhibit cardiac functional defects observed in patients and mouse models of FA, in particular heart dilatation and impaired systolic function. Here, we present further histological characterization of this model. We observed strong sarcomere alterations with loss of striation of actin fibers in cardiomyocytes of Drosophila hearts depleted for frataxin, a phenotype reversible following arrest of frataxin inactivation. The microtubule network was also disrupted and, only following strong frataxin inactivation, the mitochondrial network was deeply modified with the presence of enlarged and donut- shaped mitochondria. To identify potential therapeutic compounds, we then screened in vivo the Prestwick Chemical library, composed of 1280 compounds, on more than 20,000 flies. This screen allowed the identification of several drugs reducing the cardiac dilatation. The drug with the strongest protective effects was paclitaxel, a microtubulestabilizing drug. Thus, our results suggest that frataxin inactivation induces cardiac dysfunction through impaired sarcomere assembly and/or renewal. Considering the protective effect of paclitaxel, microtubule destabilization could be one of the mechanistic link between mitochondrial dysfunction and impaired sarcomere assembly, leading to cardiac dysfunction in FA.

86. New Drosophila models of Friedreich ataxia with GAA expansions.

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Friedreich Ataxia (FA) is caused by a GAA repeat expansion in the first intron of FXN, the gene encoding frataxin, a small mitochondrial protein, which results in decreased gene expression. Thanks to the high degree of conservation of frataxin throughout evolution, Drosophila appears as an adequate animal model to study FA disease and to evaluate therapeutic interventions. The existing Drosophila models of FA are mainly based on RNAi- mediated downregulation of fh, the fly ortholog of FXN. Here, we have generated new Drosophila models of FA, based on the insertion, in the intron of the fly fh gene, of a portion of the first intron of the human FXN gene carrying GAA triplet expansions. We observed a decrease in frataxin expression of 70% in these fh-GAA flies. Expression of a neighbor gene is also decreased, showing that the GAA expansions also affects gene expression beyond the fh locus. fh-GAA flies exhibit developmental delay and lethality. Interestingly, some individuals reach the adult stage when raised at low temperature but present strong locomotor defects, short lifespan and cardiac dysfunction. Developmental and adult phenotypes are both rescued by frataxin overexpression, showing that they are effectively due to frataxin deficiency. These new Drosophila fh-GAA models have multiple interests: they will be used to study physiopathological mechanisms involved in the disease, and to identify and evaluate therapeutic compounds, in a context that mimicks closer the situation in human patients compared to RNAi models. Moreover, they will allow to evaluate in vivo a gene therapy based on GAA deletion by the CRISPR/Cas9 system. To this purpose, we have already built genetic tools allowing inducible expression of the CRISP/Cas9 machinery ubiquitously or specifically in affected tissues.

89. Comparison of two GAA repeat expansion-based Friedreich ataxia mouse models: YG8sR and YG8LR

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Four GAA repeat expansion-based human FXN YAC transgenic FRDA mouse models have previously been characterised: Y47R (9 GAA repeats), YG8R (90/190 GAA repeats) YG22R (190 GAA repeats) and YG8sR (120-315 GAA repeats). The YG8sR model, which was derived from YG8R breeding, consists of a single copy of the FXN transgene on mouse chromosome 16. The YG8sR founder mouse contained 120 GAA repeats, but due to selective breeding, our colony of YG8sR mice now contains 215 to 315 GAA repeats, with an average length of 270 GAA repeats. YG8sR mice have significant decreases in FXN gene and protein expression, together with a progressive decline in coordination ability, in comparison to C57BL6/J and Y47R controls. Recently, the generation of a newer mouse model, designated YG8LR, has evolved from breeding of the YG8sR mice. The YG8LR founder mouse contained 410 GAA repeats, but due to selective breeding of further GAA repeat expansions our colony of YG8LR mice now contain 400 to 480 GAA repeats, with an average length of 420 GAA repeats. Through rigorous analysis by qRT-PCR, western blots, frataxin dipstick and ELISA analysis, significant decreases in frataxin gene and protein expression levels have been identified in cerebellum, heart, liver, dorsal root ganglia and spinal tissues from YG8LR mice in comparison to YG8sR mice. ELISA analysis showed a decrease of YG8LR frataxin levels by 38% in the liver and 16% in the lumbar spinal cord in comparison to YG8sR. In addition, qRT-PCR analysis of FXN levels showed a 35% decrease in the liver and a 22% decrease in the thoracic spinal cord of YG8LR mice compared with YG8sR mice. Further investigations of YG8LR and YG8sR mice are currently underway to determine the epigenetic status at the FXN transgene (DNA methylation, histone modifications and FAST-1 expression), together with behavioural and histopathological studies.

POSTER SESSION TWO

Friday 29 September
h. 5.30 – 7.00 pm
121

NATURAL HISTORY, BIOMARKERS AND ENDPOINTS

94. Systems biology approach to studies of mitochondrial dysfunction for the discovery of Friedreich's ataxia biomarkers

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C.
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Introduction: Selective cell death in neurodegenerative diseases such Friedreich's ataxia (FRDA), is thought to involve mitochondrial complex 1 dysfunction. FRDA is a devastating genetic disease resulting from elongated GAA repeats in both alleles of the FXN gene, which results in epigenetic inhibition of frataxin protein expression. There are currently no approved treatments for FRDA and there is a need for biomarkers to monitor treatment efficacy

Methods: Using stable isotope dilution liquid chromatography-mass spectrometry (LC-MS) coupled with [13C]-labeling and isotopologue analysis, the metabolic derangement in lipid metabolism in freshly isolated human platelets from FRDA patients and controls was established. In addition, highly specific LC-MS assays for serum apoliprotein A-I (ApoA-I) and tissue frataxin using stable isotope labeled
protein internal standards were employed to quantify the proteins in serum and tissue samples.

Results: There was a significant increase in labeling from [13C16]-palmitate into M + 4 for HMG-CoA in FRDA platelets (13.3 Å} 4.9%; $p < 0.001$) when compared with controls (4.8 Å} 2.6%).

This led to the analysis of serum ApoA-I and the finding that concentrations were reduced in FRDA (129.4 mg/dL serum, n=50) when compared with controls (166.6 mg/dl serum; n=50). Knockdown of the FXN gene in HepG2 cells, caused a significant decrease in ApoA-I biosynthesis. The highly sensitive LC-MS assay for frataxin (limit of detection of 5 amol/µg protein) revealed that the frataxin levels were decreased by > 50 % in the FRDA dermal fibroblasts and platelets when compared with controls

Conclusions: Overall, our systems biology approach has facilitated the discovery of in vivo biomarkers for FRDA, a disease of mitochondrial dysfunction. In view of the inverse relationship between serum ApoA-I levels and dilated cardiomyopathy, our novel finding partly accounts for the increased risk of this disease in FRDA.

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100. How does performance of the Friedreich Ataxia Functional Composite compare to rating scales?

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Introduction
Progression of Friedreich ataxia (FRDA) is often measured using neurological rating scales such as the Friedreich Ataxia Rating Scale (FARS). Performance scales comprising functional measures have been used in other conditions due to their increased sensitivity and reproducibility and may replace examination-based measures. The Friedreich Ataxia Functional Composite (FAFC) consisting of the timed 25-foot walk (T25FW), the 9-hole peg test (9HPT) and low-contrast letter acuity (LCLA), has therefore been proposed, and could be effective in assessing the progression of FRDA.
The aims of this study were to examine the relationship between the Friedreich Ataxia Functional Composite (FAFC) measures and characteristics of FRDA to determine if the FAFC is more sensitive to clinical change over time compared to the raw data of its components.
Methods
One hundred and twenty-two individuals completed all three performance measures at baseline, 63 at Year 1, 34 at Year 2 and 25 at Year 3. Composite scores called Z2 (from the combination of T25FW and 9HPT) and Z3 (from T25FW, 9HPT and LCLA) were created using methods described by Lynch and colleagues1. Correlation analyses were conducted. Change in FAFC components were examined over one, two, and three years.
Results
The FARS, Z2, Z3 and 9HPT showed significant change over all time points compared to baseline. The T25FW demonstrated significant change over three years. The LCLA demonstrated no significant change over any of the time points.
Conclusions
The FAFC shows significant change over time indicating disease progression, however this may result from individual components driving the differences rather than the robustness of the complete scale. In particular, the LCLA showed no change over time, rendering Z3 redundant. We conclude the FAFC is of limited value in cohorts with non-ambulant individuals and is therefore better suited for use in measuring change in less affected populations.
1Lynch, D.R., et al., Measuring Friedreich ataxia: Complementary features of examination and performance measures. Neurology, 2006. 66(11): p. 1711-1716.

101. Sexual function, intimate relationships and Friedreich Ataxia

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Introduction: Sexual dysfunction (SD) is reported in neurological conditions similar to Friedreich ataxia (FRDA) such as multiple sclerosis. Anecdotally individuals with FRDA report difficulty forming intimate relationships and SD including erectile dysfunction and altered genital sensation. To date there has been no formal appraisal of SD in FRDA, nor the impact SD may have on intimate relationships. This study aimed to explore if, and to what extent, people with FRDA experience challenges with sexual function and intimate relationships due to FRDA.
Methods: A questionnaire including a number of validated scales was purpose designed to explore SD and intimate relationships. Invitations to participate in the study were sent electronically to eligible participants on the Ataxia UK and Friedreich Ataxia Research Alliance databases. Responses were anonymous.
Results: One hundred and seventy-seven individuals with FRDA (female=82/150) aged 18 and over returned 118 completed and 59 partial responses. The average age of respondents was 38 years (SD: 12.9), the average age of disease onset was 17.8 years (SD: 9.5) and average score on the Functional Independence Measure was 96/112 (SD: 19.6), indicating mild functional impairment. Sixty-two percent (86/138) respondents reported that FRDA impacted on their ability to enter into intimate relationships. Fifty-nine percent of male respondents (20/34) reported a degree of erectile dysfunction and 31% (14/45) of females reported inadequate vaginal lubrication interfering with sexual responsiveness. Thirty-six percent (42/117) of all participants reported reduced genital sensation, 77% (90/117) reported problems moving their body during sexual activity and 64%, (75/117) reported reduced confidence due to FRDA interfering with sexual satisfaction.
Conclusion: This survey confirmed that FRDA impacts on the capacity to both enter into, and enjoy intimate relationships. Understanding the nature and extent of SD is critical to developing interventions and recommendations designed to enhance sexual function and intimate relationships for individuals with FRDA.

102. Keeping the black dog at bay: understanding depression in Friedreich ataxia.

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Introduction. Despite few published studies, clinical experience indicates individuals with Friedreich ataxia (FRDA) are at a significant risk of developing depression. Left untreated, depression can have a profound effect on quality of life. This study documents the incidence of depression in adults with FRDA and explores possible change in severity of depression over time.

Method. Forty-four individuals with FRDA and 44 control participants completed the Beck Depression Inventory (BDI). Of this cohort 21 individuals with FRDA completed the BDI again 24 months later. An additional cohort of 9 individuals completed the BDI on two occasions with a 10 year interval. Demographic information and clinical parameters were collected. A total BDI score of 0-13 is considered in the minimal range for depression, 14-19 is mild depression, 20-28 is moderate depression, and 29-63 indicates severe depression.

Results. The presence of mild to severe depression in 29.55% of individuals with FRDA compared to 4.55% in controls underscored a significant difference between individuals with FRDA (M=10.1; SD=9.3), and control participants (M=4.0; SD=4.3) in the total BDI score ($F(1.83) = 15.29$, $p<0.001$). However, there was no significant difference in scores on the BDI for individuals with FRDA when compared over 24 months indicating the symptoms of depression do not alter over this time period. In a smaller cohort there was a significant ($t(9)= 2.22$, $p=0.05$) difference in baseline BDI (M=10.5, SD=11.6) compared to the BDI score administered 10 years later (M=7.8; SD= 10.4) indicating a reduction in depression.

Conclusion. This study identified a significantly greater incidence of depressive symptoms in individuals with FRDA compared with controls and underscores the critical need for early screening and management of depression for people with FRDA. The absence of significant change over 24 months and improvement over 10 years suggests that depression, when present may not be progressive.

104. A unique pattern of left ventricular remodeling in Friedreich ataxia (FRDA) related to frataxin deficiency?

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Introduction: Cardiac disease is common in FRDA and is a predictor of prognosis. While FRDA cardiac involvement is often described as hypertrophy, the geometric changes in FRDA are complex, appear to be different to what is seen in hypertensive heart disease, aging and the autosomal dominant hypertrophic cardiomyopathies, and may also differ between children and adults. The aim of this study was to define the left ventricular (LV) geometric changes in FRDA.

Methods: Echocardiography was performed in 171 subjects, including 52 children (7-18 years) and 119 adults, who were homozygous for a GAA expansion in the FXN gene and had a normal LV ejection fraction. End-diastolic measurements were made of LV internal diameter (LVEDID), septal wall thickness (SWT), posterior wall thickness (PWT), LV length (LVEDL) and LV volume (LVEDV) and calculations were made of LV external diameter (LVEDED = SWT+LVEDID+PWT),
relative wall thickness (RWT = 2 x PWT/LVEDID) and LV mass (LVM).

Results: After adjustment for age, sex and BSA, GAA1 was not a correlate of SWT, PWT or LVM, but was inversely correlated with LVEDID, LVEDED, LVEDL and LVEDV ($p<0.05$ for all). After adjustment for age, GAA1 was positively correlated with RWT. Age was an independent inverse correlate of PWT, LVEDED, LVEDL, LVEDV, RWT and LVM ($p<0.05$ for all). All of the above correlations were also present in separate analysis of the adult group, but none were evident in the children.

Conclusions: In FRDA, the degree of genetic abnormality is associated with a smaller left ventricle in adults, but no direct relationship with wall thickness or LVM is evident. An explanation for the variable findings of previous studies may be the lack of similar correlations in children. Progressive LV dilatation and a survivor effect may be contributing factors to the relationship of age with smaller LV size and mass.

105. Monitoring progression of disease in Friedreich's Ataxia: a multimodal electrophysiological approach

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Background: In Friedreich's Ataxia (FRDA), a lack of functioning Frataxin manifests as a progressive neurological syndrome with features of dorsal root ganglionopathy (DRG), degeneration of the spinocerebellar tracts and corticospinal tracts (CST). Clinical trials of novel/emerging therapies that can correct the underlying molecular defect in FRDA are now planned. However, because of the low prevalence of FRDA (1/100,000) and the relative insensitivity of clinical rating scales (e.g. ICARS, FARS) at detecting treatment effects in small interventional trials (<120 patients), measures that can detect subclinical disease progression will be needed as trial endpoints.
Aim: To identify potential electrophysiological markers of subclinical disease progression.
Methods: In this preliminary study, 20 patients with a genetically confirmed diagnosis of FRDA (age 4-51 years; disease duration 6-35 years) were recruited prospectively and assessed at sixmonthly
intervals. Each assessment involved nerve conduction studies, somatosensory evoked potentials, motor evoked potentials (MEPs), Friedreich's Ataxia Rating Scale (FARS) and quantification of Frataxin levels. We also measured beta-band EMG-EMG coherence (BIMC), which is a simple, painless and non-invasive method of assessing the integrity of the CST and sensory afferents using surface EMG.
Results:
1. BIMC is absent or significantly reduced in all FRDA patients compared to controls;
2. There was a significant increase (~20%) in MEP central motor conduction time over 6 months.
Conclusion: EMG-EMG coherence is potential method of screening for the subclinical onset of disease in asymptomatic individuals with a genetic diagnosis of FRDA and identifying when such individuals should initiate disease-modifying therapy. MEPs could provide a method of monitoring subclinical disease progression in FRDA.
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106. CARFA (NCT02840669): A study to characterize the cardiac phenotype of individuals With Friedreich's Ataxia

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Cardiomyopathy occurs in more than 90% of Friedreich's ataxia (FA) patients, mostly young adults, and is severe in ~60% of them, ultimately leading to heart failure. To date, no marker has been identified to predict the onset or the severity of FA-associated cardiomyopathy and no specific therapy exists to prevent the deterioration of or to restore cardiac function. FXN gene replacement therapy constitutes a promising approach for treatment and one candidate therapy is currently under development at Adverum Biotechnologies.

A multi-parameter, prospective, longitudinal, observational clinical study has been initiated to better characterize the cardiac manifestations of the disease. Cardiac magnetic resonance imaging, echocardiography, serum cardiac biomarkers and fatigue severity are being evaluated over one year to identify markers indicative of disease progression and which could be used to measure treatment efficacy in future interventional clinical studies. In addition, a cardiopulmonary exercise test (CPET) with an arm-bicycle ergometer has been specifically designed for FA patients, with the objective of testing the feasibility, reproducibility and reliability of this test as a functional evaluation of FA at different stages of the evolution of the disease.

At the time of submission of this abstract, 15 FA patients diagnosed with FA-associated hypertrophic cardiomyopathy (10 males, 5 females) and 9 age and gender-matched healthy volunteers have been enrolled in the study (target enrollment: 20 FA patients and 20 healthy volunteers). Average age of FA patients is 30 years old (range 24-47), average duration from first symptom is 13.2 years (range 5-27), and 9/15 patients (60%) are ambulatory. Average SARA score was 20/40 (SD 7.9). Most patients carried two GAA expansions with a shortest allele range of 100 to 800 (mean 464). Two patients were compound heterozygous carriers of a GAA expansion and a point mutation.

Baseline data will be presented.

108. Ambulatory status and quality of life in children with Friedreich ataxia

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Background: Friedreich ataxia (FRDA) is a recessively inherited neurodegenerative disorder, often of childhood onset. The majority of patients present with ataxia and mobility devices are the mainstay of managing loss of ambulation due to progressive ataxia and muscle weakness.

Objective: To determine the impact of loss of independent ambulation and use of a mobility device on quality of life (QOL) scores in children with FRDA.

Methods: Participants were 111 individuals from 9 institutions with genetically confirmed FRDA, who were less than 18 years of age. Data was collected as part of a prospective natural history study and standardized clinical evaluations were carried out across all study sites, including health related QOL using the PedsQL 4.0 Generic Core Module as the main outcome measure. The association between ambulatory status/use of mobility device and QOL scores was evaluated using univariate and multivariate regression. Longitudinal QOL data available for 16 individuals who transitioned to or between mobility devices was also analyzed.

Results: Mobility device use was associated with worse mean PedsQL total scores and worse mean physical, emotional, social, and academic subscores, after adjusting for gender, age of disease onset, and total Friedreich Ataxia Rating Scale (FARS) score. The magnitude of the difference was greatest for the physical subscore (19.5, p=0.0004) and least for the emotional subscore (10.61, p=0.031). Transition to or between a mobility device was associated with a worse mean physical subscore (18.13, p=0.012); there were no statistically significant changes in emotional (10.4, p=0.19), social (2.66, p=0.71) or academic subscores (4.0, p=0.6), and there was a trend toward improvement in emotional subscore over time (9.55 per year, p=0.06).

Conclusions: Loss of independent ambulation has a significant impact on QOL in children with FRDA, but appears to affect the physical domain to a much greater extent than psychosocial health.

110. Healthcare practices and socio-economic impact in Friedreich's Ataxia

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Introduction: The severity of clinical symptoms and progressive nature of Friedreich's Ataxia (FA) are likely to have significant socio-economic and healthcare consequences. This study aimed to understand the actual impact of FA on socio-economics and healthcare received by disease severity.

Methods: DeNovo, Horizon, and FARA developed a 47-question survey exploring, demographics and socio-economics, disease severity (ambulatory status), current treatments and supportive care received, and other healthcare practices. DeNovo fielded the online survey which took respondents an average of 40 minutes to complete. FARA recruited a representative mix of FA patients and caregivers enrolled in their U.S. Registry of 1122 individuals. Data were analyzed by total respondents and by ambulation status groups.

Results: 203 FA patients or caregivers completed the survey, with a nearly equal distribution of individuals being ambulatory and non-ambulatory. Neurologists are identified as the 'main' diagnosing and treating physicians. The average time from initial physician visit to FA diagnosis was 2-4 years; an average of 3-4 HCPs were seen prior to diagnosis. 53% of FA patients had either Medicare or Medicaid health insurance. Fewer than 20% of adults with FA were employed full-time; median employment time was 7 years. 86% of patients reported needing some help with Activities of Daily Living (ADLs). 65% were determined disabled by the Social Security Administration by the age of 30 years and 30% received Supplemental Security Income.

Conclusions: The results provide significant insights into the healthcare practices and socioeconomic
implications of FA. The time to FA diagnosis and referrals through multiple HCPs reflect the continued need to raise awareness. While individuals with FA are able to achieve high levels of education and professional training, employment time is limited. Most adults require assistance with ADLs and rely on government program support for health insurance and income, reflecting the profound physical effects of FA.

114. Baseline disease severity predicts longitudinal brain atrophy over 2-Years in Friedriech Ataxia: the IMAGE-FRDA Study

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Introduction: Cross-sectional magnetic resonance imaging (MRI) studies of brain structure in Friedreich Ataxia (FRDA) report atrophy in the cerebellum, subcortical nuclei, and frontal cortices. This study aimed to determine whether MRI could also detect longitudinal volume change over a 2-year period in a large FRDA cohort.
Methods: Structural MRI was acquired at two time-points (2.02Å}0.14 years apart) in 28 individuals with FRDA and 29 healthy controls. Rate of volume change across the whole brain was calculated using the SPM12 Longitudinal Registration Toolbox. Non-parametric permutation tests, with family-wise error (FWE) cluster-corrected thresholds, were used to infer between-group differences and associations with baseline disease severity (Friedreich Ataxia Rating Scale (FARS); cohort range:19-126).
Results: Significantly greater volume loss in FRDA, relative to controls, was evident in the white matter of the midbrain, internal capsules, and splenium of the corpus callosum (pFWE<0.03), and at trend levels in the superior cerebellar peduncle and vermal grey matter (pFWE=0.086). In the FRDA cohort, significant correlations with baseline FARS indicated that diffuse cerebellar grey matter atrophy occurred maximally in individuals at earlier stages of the disorder (FARS<~60), followed by later relative stability (r=0.56, pFWE=0.019). Conversely, in the grey and white matter of the sensorimotor and premotor cortices, volume gain was evident in early disease (FARS<~60), with later volume loss in these regions (FARS>~100; r=-0.66, pFWE=0.003).
Conclusions: MRI is sensitive to progressive brain atrophy in FRDA over a 2-year period. This atrophy occurs most consistently in the white matter of the cerebellar-thalamic tracts and corpus callosum, consistent with reports of longitudinal microstructural degradation in these same regions. In contrast, disease state plays an important moderating role in patterns of cerebellar and cerebral cortical atrophy, providing insights into the sequelae of the underlying neuropathology. These results support the potential utility of structural MRI as a biomarker of FRDA progression, which may be optimised by accounting for individual disease severity.

124. Body Mass Index and Stature in the Friedreich Ataxia Clinical Outcome Measure Study

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Objective: Friedreich Ataxia (FA) is a progressive neuromuscular disorder. Anthropometric measures reflecting weight relative to height (body mass index, BMI) and stature (height) are important indices of health. The objective of this study was to identify factors associated with BMI and stature in FA.

Research Design and Methods: Participants were 769 individuals from 12 international sites in a prospective natural history study (FA Clinical Outcome Measure Study, FACOMS). For this cross-sectional analysis, we used the first visit where simultaneous height and weight measurements were obtained. Age- and sex-specific BMI and height z-scores were calculated using CDC 2000 growth standards for participants <20y. For adults ≥20y, the reference range for 20 year-olds was used to permit comparisons. Detailed clinical information was available (n=471). Multivariable linear regression analyses were used to investigate the associations between factors and outcomes.

Results: Median age of participants was 21.9y (IQI, 14.4-33.6y); 50% (n=379) were female. The shorter GAA repeat length was a median of 680 bp (IQI, 500-800, n=728 with genetic information). 4.8% had point mutations in FXN. 7.3% had diabetes mellitus (DM), 82% had scoliosis, and 60% had cardiomyopathy. Increasing age (b=0.024; p<0.001) and having a point mutation (b=0.46; p=0.042) were each positively associated with BMI-z. When clinical conditions were added to the model, scoliosis had an independent negative association with BMI-z (b=-0.40; p=0.016). In adults only, increased GAA repeat length (b=-0.003; p=0.05) or having scoliosis (b=-1.67; p=0.013) were negatively associated and having DM (b=2.19; p=0.027), or a point mutation (b=4.89; p=0.006) was positively associated with BMI (kg/m2). Increased GAA repeat length (b=-0.0004; p=0.030) is negatively associated and age (b=0.007; p=0.037) is positively associated with relative height.

Conclusions: Anthropometric measurements are independently associated with both genetic and clinical factors, and may reflect overall health in FA. In future, longitudinal analyses will yield important additional insights.

125. Acute effects of dietary glycemic index on lactate and glucose homeostasis in individuals with Freidreich's Ataxia and other disorders affecting mitochondria

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Center for Advancing Translational Sciences (NCATS): UL1TR000003, UL1TR001878. We appreciate assistance with recruitment from the Freidreich's Ataxia Research Alliance (FARA) and North American Mitochondrial Disease Consortium (NAMDC). We also appreciate Dr. Belinda Lennerz sharing technical details regarding test meal preparation.
Introduction: Individuals with Friedreich's Ataxia (FA) and other disorders affecting the mitochondria are at increased risk of diabetes mellitus, a condition for which nutrition plays a critical role in management. The "glycemic index" (GI) of a food refers to its tendency to raise blood glucose. The objective of this study was to test the effect of low- versus high-GI meals on glucose and lactate homeostasis and cognition in individuals with mitochondrial diseases.
Methods: We performed a randomized, double-blinded, crossover study in adults (n=17) with primary mitochondrial disorders (NCT02284334). On two separate occasions, in random order, participants received a low-GI (37/100) or a high-GI (84/100) test "shake", with identical proportions of energy from carbohydrate (59%), protein (15%), and fat (26%). Measurements were made every 30 minutes for 4 hours of blood glucose and lactate. Cognitive testing (computerized test of sustained attention and vigilance) was administered 2 and 4 hours after each meal.
Results: Ten of 17 participants had FA. 15/17 participants completed both visits. 52% (9/17) were female, mean age was 40 years (SD 14, range, 18 – 62). After the high-GI meal, maximum blood sugar was 150 mg/dL, versus 125 mg/dL after the low-GI meal (p=0.00075, mixed effects regression analysis). After the high- GI meal, 61% of participants experienced a late blood sugar decrease to <70 mg/dL, versus 14% after the low- GI meal (p=0.0052, Fisher's exact test). Using mixed-effects multivariate linear regression analysis, we found a 14% reduction in lactate area under the curve (AUC) following the low-GI meal as compared to the high-GI meal (SE = 6.5%, p=0.047, accounting for age). With respect to cognitive testing, at the 4-hour time point only, sustained attention (ability to continuously discriminate targets from non-targets) was worse during the high-GI meal (unadjusted p-value=0.03, by paired t-test).
Conclusions: Low-glycemic index sources of nutrition may offer advantages in individuals with FA. The role of nutrition in the management of conditions affecting the mitochondria should be the focus of future study.

126. Motor GABA levels predict clinical impairment in children with Friedreich ataxia

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The objective of this study is to observe the relationship between estimates of GABA levels, derived from edited magnetic resonance spectroscopy (MEGAPRESS-MRS), and clinical scores of impairment in children and adults with Friedreich Ataxia (FRDA). FRDA is debilitating lifeshortening
degenerative neuro-muscular disorder which presents in childhood and leads to progressive ataxia (e.g., lack of muscle coordination affecting speech, sensory and motor impairments of the limbs etc.). While no cure exists, several potential therapies are under development. Direct measurement of the relevant neurophysiologic parameters is necessary in order to understand the specific effects of different therapies on the pathways involved in FRDA. Our pilot work suggests that MRS GABA/Cr concentration from primary motor regions may yield correlates of impairment shown in FRDA.

Spectrally-edited MEGAPRESS (TE 80ms) MRS from left-hemisphere MI 3x3x3 cm3 voxels were obtained in a cross-section of children and adults with FRDA ((N=13; age 11.3 to 34.6 years). Clinical measures of impairment included the Friedreich Ataxia Rating Scale (FARS) battery which includes: timed 25 foot walk, 9-hole peg test, low contrast letter acuity, quality of life measures, ataxia disability score, and ataxia activities of daily living (ADL). Pearson correlation of the GABA/Cr value and corresponding FARS score shows a non-significant trend $p<0.11$. Future work will attempt to determine 1. The degree to which these scores are due to changes in cortical thickness within the prescribed GABA voxel 2. The functional (neural and behavioral) correlates of GABA/Cr downregulation in motor regions in FRDA.

Figure 1: MRS Motor voxel GABA. Left: GABA concentration from primary motor cortex (MI) is associated with Friedreich Ataxia Rating Scale values (FARS; large numbers = greater impairment). This demonstration of MI GABA predicting baseline FARS scores represents a novel and promising biomarker for gene therapy.

127. Biomarkers in FRDA cardiomyopathy to monitor disease progression

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Background/Hypothesis: A clinical hallmark of Friedreich's Ataxia (FRDA) is severe cardiomyopathy, which is the most frequent cause of death in FRDA patients (1). Treatments for FRDA cardiomyopathy are limited by our inability to monitor changes in disease progression with therapeutic intervention. Thus, we measured protein biomarkers coupled with metabolite profiling to identify serum markers that accurately reflect the stages in FRDA cardiomyopathy progression.

Methods: 5 controls and 11 FRDA patient serum samples were provided by Dr. Mark Payne with clinical information (e.g. parameters: clinical profiling, electrocardiography, EKG; echocardiography, ECHO). We used mass spectrometry to identify acylcarnitines (ACs) and amino acid (AAs), and ELISA multiplex technology to identify protein metabolites. Mean difference statistical analysis was used to identify parameters with significant difference between controls and FRDA patients. These select parameters were then used as the predictor variables in our linear regression models. To determine statistical significance, Bonferroni and Benjamini- Hochberg adjustments were performed at level $\alpha = 0.05$, $\alpha = 0.25$ and $\alpha = 0.50$.

Results/Conclusions: In addition to the EKG and ECHO measures identified as predictor variables, we also used the FRDA-specific parameters age of onset and repeat length. In contrast to the literature, repeat length (long or short allele) poorly correlated with the other clinical parameters and the response variables. Cardiac-specific measures EKG measures and ECHO left ventricular wall thickness demonstrated a strong ($\alpha = 0.05$), positive correlation with even- chained acylcarnitines (ACs). This indicates increased ACs with left ventricular hypertrophy and abnormal shifts in cardiac conduction. These findings are consistent with the literature which describes a strong association between elevated serum even-chained ACs and increased risk of cardiovascular death in patients with stable angina pectoris (2).

Future Studies: We are currently conducting a two-stage analysis, validating our findings with more serum collections from new patients.

1. Koeppen AH, Becker AB, Feustel PJ, Gelman BB, Mazurkiewicz JE. The significance of intercalated discs in the pathogenesis of Friedreich cardiomyopathy. Journal of the Neurological Sciences 2016;367:171–176.

2. Strand E et al. Serum Acylcarnitines and Risk of Cardiovascular Death and Acute Myocardial Infarction in Patients With Stable Angina Pectoris. Journal of the American Heart Association 2017;6(2):e003620.

128. Correlation between GAA expansion length and frataxin upregulation in Friedreich's ataxia

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Introduction: Friedreich's ataxia (FRDA) is an autosomal recessive neurodegenerative disorder resulting from an unstable GAA repeat expansion situated in intron 1 of the frataxin (FXN) gene, which encodes the mitochondrial protein frataxin (FXN). This pathogenic expansion causes epigenetic silencing of FXN resulting in a reduced production of frataxin in patients leading to the FRDA disease phenotype. Specifically, the size of the shorter expanded allele is inversely correlated with disease onset and progression, where late-onset and slower progressing cases are associated with smaller GAA expansions. Various compounds have been shown to upregulate the levels of FXN in FRDA, either in vivo or in vitro, including several histone deacetylase (HDAC) inhibitors and vitamins. We identified four compounds (Nicotinamide, Thiamine, BIX-01294, benzamide HDAC inhibitor 109) that although have been proven to upregulate FXN, have reported variable efficacy, which has been suggested to be due to different expansion lengths in FRDA patients. Therefore, we investigated the correlation between the efficacy of these compounds in upregulating FXN and different GAA repeats expansion lengths present in FRDA patients.
Methods: To this purpose, peripheral blood mononuclear cells (PMBCs) were extracted from several FRDA patients and controls. PMBCs were treated with each compound. FXN mRNA and protein levels were assessed in untreated and treated PBMCs via qPCR and Western Blot. To determine the length of GAA repeats expansion, long-range PCR was applied.
Results: Based on previous investigations and initial data we report that the pathogenic expansion length in FRDA patients appears to have an inverse correlation with FXN upregulation.
Conclusions: Our findings demonstrate that future treatments aimed at upregulating frataxin in FRDA should be determined by personalised medicine based on the genetic profile of individual patients.

129. Targeted quantitation of coenzyme A metabolites and serum apolipoprotein A-I by LC-MS for monitoring mitochondrial dysfunction in Friedreich's ataxia

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Introduction: Friedreich's ataxia (FRDA) is associated with decreased levels of frataxin mRNA and mature frataxin protein. There is increasing evidence from studies with frataxin-deficient cells, mouse models and human tissues that this results in mitochondrial dysfunction together with changes in fatty acid oxidation and lipid metabolism.
Methods: Stable isotope dilution liquid chromatography-mass spectrometry (LC-MS) coupled with [13C]-labeling and isotopologue analysis were used to characterize the metabolic derangement in lipid metabolism in FRDA platelets. Highly specific and accurate LC-MS assay for serum apolipoprotein A-I (ApoA-I) using a stable isotope labeled protein internal standard was employed to quantify ApoA-I in serum samples from FRDA patients.
Results: A significant decrease in labeling from [13C6]-glucose to into M+2 for 3-hydroxy-3-methylgutaryl-coenzyme (HMG-CoA, an intermediate in the mevalonate and ketogenesis pathways) was revealed in FRDA platelets (10.3 Å} 7.3%, $P < 0.05$) when compared to controls (16.7 Å} 4.8%). In contrast, there was a significant increase in labeling from [13C16]-palmitate into M+4 for HMG-CoA in FRDA platelets (13.3 Å} 4.9%, $p < 0.001$) when compared to controls (4.833Å} 2.6%). These metabolic changes led us to analyze serum Apo A-I, which revealed that levels
were reduced in FRDA (129.4 Å} 26.0 mg/dL, n=50) when compared to controls (166.6 Å} 37.2 mg/dL, n=50). Experiments conducted in FXN knock-down HepG2 cells, showed a significant decrease in ApoA-I biosynthesis. A pilot study of statins in FXN knock-down HepG2 cells also revealed an increase of ApoA-I level along with decreased HMG-CoA.
Conclusions: Targeted quantitation of platelet acyl-CoA thioester metabolites and serum ApoAI by LC-MS has provided biomarkers for monitoring the treatment of mitochondrial dysfunction in FRDA. The reduced serum ApoA-I levels found in FRDA can potentially be normalized by statin treatment.
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130. Living with Ataxia in Ireland 2016–a nationwide survey of 130 Irish patients with inherited Ataxia

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Introduction
Background: In Ireland a handful of neurologists manage the majority of people with inherited ataxia. With little information in the literature and with the support of the national patient organisation people with different types of ataxia were asked to complete a comprehensive survey to evaluate disability, resource use and QoL.
Objective: To collect real-life data from a large cohort of patients with inherited ataxia in Ireland, with special attention to the individual ataxia-related healthcare resources and costs, disability due to the disease and quality of life (QoL) measures. We aimed to compare responses of patients from a heterogeneous population across groups of all ages, different underlying genetic causes and disease durations.
Methods
Over 250 anonymous surveys were distributed in clinics, by post and at ataxia meetings nationwide. We report observational descriptive data and compare groups using nonparametric statistics.
Results
One hundred and thirty patients (45% males) responded. Seventeen percent were <25 years of age, 31% were >60 years old. Nine percent were working full or part time, 47% were unable to work or retired early due to ataxia. Thirty-nine percent were wheelchair-bound. Fifty-nine percent had symptom onset <20 years, 23% had late onset >40 years. As expected, the majority (42%) had Friedreich's ataxia, 28% did not have genetic diagnosis. Forty-five percent relied on professionally paid care. Group comparisons and QoL data will be reported.
Conclusions
To date this is the first study in Ireland and the largest single-country 'real-life' patient survey in Europe looking at patients with various types of inherited ataxia with comprehensive data on disability, healthcare resource use and QoL.

131. Longitudinal change of gait and balance in individuals with Friedreich ataxia

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Introduction: Gait ataxia and instability are common presenting features of Friedreich ataxia (FRDA). Mobility declines with disease progression until ambulation is no longer possible, approximately 10- 15 years following initial symptoms. This study aims to determine valid and responsive gait and balance outcome measures to detect progressive change over 12 months for individuals with FRDA. Methods: Forty-two individuals with FRDA underwent assessment at baseline and six months (12- month data is being collected). Measures included (i) gait parameters at preferred and fast speeds using the GAITRite. instrumented walkway, (ii) Biodex Balance SystemTM SD postural stability test (PST) and limits of stability (LOS), (iii) Berg Balance Scale (BBS), (iv) Timed 25 Foot Walk Test and (v) Dynamic Gait Index. Correlations between objective measures, the Friedreich Ataxia Rating Scale (FARS) neurological exam, Scale for the Assessment and Rating of Ataxia (SARA) and disease characteristics were examined. The standardised response mean was reported as the effect size index for comparison of internal responsiveness. Results: Significant correlations were found between the BBS and SARA ($p<0.001$). A lower LOS score was associated with earlier disease onset ($p=0.002$) and a higher FARS score ($p<0.001$). The SARA and FARS did not detect a significant change over six months. However, the BBS significantly decreased with an effect size index (ES) of -2.36 ($p=0.026$) over this time period. Similarly, the PST anterior-posterior index with eyes-closed detected balance decline (ES=1.00, $p=0.026$). A significant decrease in normalized stride length during fast walking was also evident (ES=-0.54, $p=0.040$). Conclusions: The BBS, PST anterior-posterior index with eyes-closed and normalized stride length are more sensitive to the decline in individuals with FRDA as compared to previously validated measures of disease severity, the SARA and FARS. These gait and balance measures may provide sensitive, objective, and clinically meaningful measurements for use in clinical trials and therapeutic inventions.

133. The autonomic nervous system in FriedreichÅLs Ataxia: preliminary findings

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Introduction: FRDA is a hereditary neurodegenerative disorder characterized by progressive gait ataxia, dysmetria and dysarthria. Further features are hypertrophic cardiomyopathy and diabetes mellitus. The disease is caused by an intronic GAA expansion in the FXN gene. Age at onset and disease severity strongly correlates with the length of the shorter GAA repeat (GAA1). The neurodegeneration in FRDA primarily involves the dorsal root ganglia and large myelinated nerve fibers in peripheral sensory nerves. Recently an involvement of small unmyelinated fibers has been demonstrated in skin biopsy of FRDA and it has been correlated with disturbances in temperature and pain perception. The presence of autonomic correlates was not investigated yet in genetically confirmed cases.

Methods: Eighteen genetically confirmed FRDA patients were consecutively enrolled. Each patient underwent a laboratory work-up and echocardiography to rule out diabetes and cardiomyopathy respectively. Disease severity was quantified through the SARA scale. Autonomic function was investigated by means of a cardiovascular tests battery (head-up tilt, active standing, Valsalva maneuver and deep breathing), the skin sympathetic reflex and the SCOPA-aut autonomic questionnaire.

Results: Mean age at examination was 40Å}14 years and mean SARA score was 22. The average GAA1 repeats were 500Å}310 and mean disease duration was 19Å}10 years. Two patients had impaired glucose tolerance and were excluded from autonomic evaluation. Abnormal autonomic findings were found in 8 patients (50%). Two out of 10 patients (20%) had delayed orthostatic hypotension, while 3/10 (30%) and 4/12 (33%) had abnormal Valsalva and Deep Breathing ratio respectively. Skin sympathetic reflex was absent in 4/12 patients (33%). The mean SCOPA-AUT was 12 (range: 3-27). No difference regarding age at examination, GAA1 repeats, disease duration and severity, was found in the comparison between the patients with or without abnormal autonomic findings.

Conclusion: We observed subtle alterations of autonomic function in our FRDA cohort independently from disease severity. That could be attributed to multiple determinants as well as to an impairment of peripheral autonomic relays.

134. Peripheral blood gene expression biomarkers in Friedreich's ataxia patients

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Introduction: Advances in the understanding of disease pathology in Friedreich's ataxia (FRDA) have led to the development of a large number of candidate treatments. However, evaluating the effectiveness of these treatments is challenging because the disease progresses relatively slowly and clinical scales lack the sensitivity to identify short term changes in disease state. The purpose of this study is to find biomarkers in peripheral blood gene expression which can serve as more precise quantifications of disease state in FRDA patients.
Methods: RNA was extracted from peripheral blood from 409 FRDA patients, 226 carriers and 90 unaffected controls. Gene expression was quantified using Illumina HT-12 v4 microarrays. Differential expression analysis was used to identify genes whose expression was significantly different between diagnostic groups, and linear regression was used to identify genes which were correlated with shorter repeat length in patients (GAA1). Network analysis (WGCNA) was used to identify modules of co-expressed genes associated with diagnostic group or GAA1. Machine learning was also used to identify a set of genes which predicted diagnostic group (classification) or GAA1 (regression).
Results: 215 genes were found to be differentially expressed between patients and controls, and 669 genes between patients and carriers. 368 genes were found to be significantly positively or negatively correlated with GAA1. Network analysis identified two modules were significantly different in patients and one module was found to be positively correlated with GAA1. Finally, classifiers for patient vs. control and patient vs. carrier identified 243 genes and 466 genes, respectively, which predicted diagnostic class with high accuracy. The predictive regression model for GAA1 identified 277 genes which predicted GAA1 with low error.
Conclusions: A large number of peripheral biomarkers for FRDA were identified that will be helpful in quantifying disease state and treatment effectiveness.

136. Cortical responses and change detection to auditory and somatosensory stimuli in Friedreich ataxia

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Introduction

Neurophysiological assessment of the proprioceptive and cerebellar systems, whose dysfunction and degeneration underlie the afferent and cerebellar ataxia characterizing Friedreich ataxia (FRDA), may define the severity and timing of their involvement, guide the identification of therapeutic targets, and provide biomarkers reflecting disease status, progression and response to treatments.

Previous studies on evoked potentials (EPs) in FRDA proposed that impairment in somatosensory EPs (SSEPs) correlates with GAA1 and does not change with disease progression, while impairment in brainstem auditory EPs (BAEPs) was reported to correlate with disease duration, suggesting an early and stable deficit in somatosensory processing and a progressive involvement of the auditory system. However, a limitation of SSEP studies is the complete loss of these responses in most FRDA subjects, even at a young age, a finding we could confirm with our patients.

Methods

We used magnetoencephalography (MEG) to study cortical evoked responses of FRDA subjects to somatosensory and auditory stimuli, with the assumption that this technology might allow to detect responses even when traditional EPs cannot be measured, and add temporal and spatial resolution to the analysis. We also explored MEG signals generated by sensory change detection, which are thought to be modulated by the cerebellum. In traditional EP protocols, an increased evoked response, called mismatch negativity (MMN), is observed when a deviant stimulus occurs amongst a sequence of repeated standard stimuli. MMN is the correlate of pre-attentional change detection in sensory cortices. An equivalent signal can be measured by MEG. Of notice, unilateral cerebellar lesions lead to near absent MMN for ipsilateral deviant somatosensory stimuli, but have no effect on auditory change detection.

We studied 16 FRDA patients (10 females, 6 males), with a mean age of 30 years (range 9-53) and a mean SARA score of 23.4 (range 9.5-37.5), and 16 healthy controls (9 females, 7 males), with a mean age of 29 years (range 10-55). We recorded whole-scalp MEG (Elekta, Oy) while undergoing (1) a tactile oddball paradigm where standard stimuli consisted of pneumatic stimulation of the right forefinger fingertip and deviant stimuli of simultaneous stimulation of the first two phalanxes; and (2) a monaural auditory oddball paradigm where standard stimuli consisted of audible tones of 540 Hz and deviant stimuli were 600 Hz tones, presented in the right hear. Inverse modelling was done using the Minimum Norm Estimate (MNE). For group analysis, individual source power time series were normalized by the maximum amplitude of standards responses before group averaging, to exclude individual subjects' amplitude effect. We temporally realigned time series on the first peak activation to control for individual responses latencies. We used non-parametric permutation statistic tests to assess significance of evoked responses.

Results

Cortical somatosensory evoked responses were found in all subjects at left primary somatosensory cortex (SI). In FRDA subjects their mean latency was significantly longer (53 vs 28 ms; $p<0.001$), and their mean amplitude was significantly smaller (0.285 vs 0.513; $p=0.0041$) than in controls. GAA1 negatively correlated with the amplitude of individual SI responses ($r=-0.74$, $p=0,0032$)

Cortical auditory evoked responses were found in all subjects at primary auditory cortex (AI), bilaterally. In FRDA patients their mean latency was significantly longer than in controls (107 vs

87 ms; $p<0.001$), but their amplitude was comparable in FRDA and controls (0.507 vs 0.45; $p=0.25$). GAA1 negatively correlated with individual AI responses ($r=-0.56$, $p=0.036$) of FRDA subjects.
Larger amplitude responses to deviant stimuli, the MEG equivalent of MMN, were found in controls and FRDA patients at left secondary somatosensory cortex (S2), with a delay of 100-200 ms; but also at left S1 in FRDA patients, with a delay of 50-78ms.
The normalized magnitude of deviant stimuli responses was significantly smaller for FRDA patients, and negatively correlated with GAA1 ($r=-0.6$, $p=0.023$).
Similarly, responses to deviant auditory stimuli were found in patients and controls over the left superior temporal lobe, with a delay between 150-200ms and comparable normalized magnitudes for both groups.
Conclusions
MEG allows to detect cortical responses to tactile stimuli in all FRDA patients, even when SSEPs are absent. These responses are delayed and reduced in amplitude. Cortical auditory responses are not decreased in amplitude, but show increased latency. In both cases, impairment is seemingly unrelated to disease progression and only correlates with mutation severity, indicating that these parameters are biomarkers of early sensory damage. Cortical responses to deviant somatosensory stimuli (corresponding to MMN) are normally measured at S2 only, as it was the case with our controls, but in FRDA subjects they occurred in S1 as well.

141. MR imaging of the spinal cord and brain in Friedreich's Ataxia

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Introduction:
Friedreich's ataxia (FRDA), the most common inherited ataxia, is a spinocerebellar neurodegenerative disorder. Neuronal loss mainly affects the spinal cord, medulla and cerebellum, but also supratentorial cortical areas. The aim was to evaluate the extent of both spinal cord and brain degeneration in FRDA using magnetic resonance imaging (MRI).
Methods:
21 genetically confirmed FRDA patients (mean age 35.0Å}12.2 years, 10 male) and 21 age- and gender-matched healthy controls (34.4Å}12.0 years, 10 male) underwent 3T MRI of the spinal cord and brain using 3-dimensional T1-weighted gradient-echo sequences. Semiautomatic segmentation procedures were applied to measure the cross-sectional area and volume of each section of the cervical and thoracal spinal cord (C1-C7, T1-T10), as well as of volumes of the brainstem, cerebellum, and cortical lobes. Associations of volumetric data with ataxia severity (Scale for the Assessment and Rating of Ataxia, SARA) and ataxia behavioral measures (Spinocerebellar Ataxia Functional Index, SCAFI) were assessed using partial correlations adjusted for age.
Results:
The entire spinal cord in patients with FRDA was flattened showing large effect sizes compared to controls (cervical: Cohen's d = 0.87 [C7] to 1.75 [C3], thoracic: 0.82 [T10] to 1.90 [T2]). For brain measures, most pronounced atrophy was observed in the medulla (d=1.80), pons (d=0.91), midbrain (d=1.06), and cerebellar white matter (d=0.86). Cortically, we found volume reductions in frontal (d=0.87) and occipital lobes (d=0.76). While both spinal data and cerebellar white matter correlated with SARA (r=-0.47 to -0.56), brainstem and cerebellar volumes were also associated with SCAFI performances (9-hole-peg-test: - 0.46 to -0.64).
Conclusions:
Our results demonstrate the extent of atrophy not only of the spinal cord, but also of the cerebellum and brainstem associated with different aspects of disease severity in FRDA. Thus, if confirmed in longitudinal studies, both spinal and brain MRI may serve as surrogate endpoints in future clinical trials.

142. Structural signature of classical vs late-onset Friedreich's ataxia by multimodality brain MRI

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Introduction: Friedreich's ataxia (FRDA} is the most common autosomal-recessive ataxia worldwide. It is clinically characterized by sensory abnormalities, slowly progressive ataxia and early onset, mostly in childhood and adolescence. However, there is a sub- group of patients with FRDA that manifest the disease after the age of 25 years and is classified as late-onset FRDA (LOFA). Therefore, we propose a transversal multimodal MRI-based study to investigate which anatomical substrates are involved in the classical (cFRDA) and LOFA.
Methods: We enrolled 36 patients (13 with LOFA) and 29 healthy controls. All subjects underwent magnetic resonance imaging in a 3T device, three-dimensional high resolution T1-weighted and diffusion tensor images were used to assess gray (GM) and white matter (WM) respectively. We used T1 multi-atlas approach to assess deep GM and thickness measures to evaluate cerebral cortex. We also used DTI multi-atlas approach to assess WM. All analyses were corrected for multiple comparisons.
Results: Group comparison showed that in both groups there was GM atrophy mostly in the motor cortex. Regarding WM, we found abnormalities in the cerebellar peduncles, pyramidal tracts, midbrain, pons and medulla oblongata for both groups, but the microstructural abnormalities in the cFRDA group were more widespread and severe. However, we found that the corticospinal tract presented more severe microstructural damage in the LOFA group. In addition, the midbrain volume of the cFRDA group correlated with disease duration ($R=-0.552$, $p=0.012$) and severity ($R=-0.783$, $p<0.001$).
Conclusion: The cFRDA and LOFA have similar, but not identical neuroimaging damage pattern. The corticospinal tract showed more severe compromise in the LOFA group, which is in line with the more prominent pyramidal signs found in these patients. Midbrain volume is a promising neuroimaging biomarker for clinical trials in cFRDA patients.

145. Analysis of correlations among four measures of disease progression in Friedreich's ataxia.

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INTRODUCTION: Friedreich's ataxia (FA) is a neurodegenerative disorder characterized by cardiomyopathy; approximately two-thirds of patients with FA die from cardiac causes. FA is commonly assessed primarily using the Friedreich Ataxia Rating Scale (FARS), a subjective neurological measure. Alternatively, timed 25-foot walk (T25FW), peak VO2, and peak workload measures are considered. These three alternative measures are compared with FARS scores to determine whether they reflect FARS and thus reflect FA progression.

METHODS: Baseline data were taken from a phase I clinical trial in which 19 patients with FA were administered the FARS Neurological, the T25FW test, and a recumbent exercise bike test for peak workload and peak VO2. Spearman's rank correlation coefficients on the FARS scale, the inverse of T25FW time (T25FW-1), peak workload, and peak VO2 were calculated. Linear regression was used to approximate the rates of decline of the measures as a function of FARS.

RESULTS: The FARS Neurological scores negatively correlated with peak workload (R=0.82), peak VO2 (R=0.60), and T25FW-1 (R=0.91). Increasing FARS Neurological score by 3 points corresponded with a 0.15 points decline in log peak workload, a 0.09 points decrease in peak VO2, and 0.012 points decrease in T25FW-1.

CONCLUSION: The three alternative measures correlated highly with FARS increase and should be considered as an addition to or substitution for FARS as they also measure cardiopulmonary and skeletomuscular decline. A study on exercise capacity in children and adolescents with FA found similar correlations between peak workload and FARS (R=0.64), and peak VO2 and FARS (R=0.46). Replicating this study longitudinally, in a larger population, is suggested, in which correlations should be observed in sub-stratified populations.

146. Cognition in Friedreich Ataxia: a neuropsychological and RS-FMRI study.

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Introduction
Several studies have evaluated cognitive impairment in Friedreich Ataxia (FRDA) reporting a modest and discordant cognitive dysfunction. Previous activation fMRI studies showed the presence of low activation patterns during motor and behavioral tasks. Unfortunately, no resting- state fMRI (RS-fMRI) analysis has been performed in FRDA.
Methods
We tested FRDA patients and sex, age, and education matched controls with an extensive neuropsychological battery. All MRI studies were performed on the same 3 Tesla scanner. For each subject, BOLD signal time course was calculated over 44 different regions, chosen because linked to the specific tested cognitive functions. The resulting functional connectivity (FC) maps were entered in a second level analysis to test for differences between the two groups. Differences were considered significant for $P<0.0011$, corrected for multiple comparisons.
Results
We enrolled 24 FRDA patients and controls. Neuropsychological tests showed impairment in all areas except for language, intelligence, and some memory tests. Nineteen patients and twenty controls were enrolled in the RS-fMRI study. Two were excluded because of motion artifacts. For the remaining 37 studies, clusters of significant difference in FC between the two groups were observed for the following tested regions: the right (r_PaCiG) and left paracingulate gyri (l_PaCiG), the right superior frontal gyrus (r_SFG), the right medial frontal gyrus (r_MFG) and the left middle temporal gyrus (l_MTG).
Conclusion
FRDA showed a worst than expected and diffuse cognitive impairment with widespread alterations of FC. The paradigm of FRDA patients being cognitively normal should be revised in favor of a non-demented, but diffusely impaired phenotype.

147. Normalization of timed neuropsychological tests with the PATA rate and nine-hole pegboard tests

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Introduction: Despite neurological patients show frequent physical impairment, timed neuropsychological tests do not take this into account during scoring procedures. We propose a normalization method based on the Pata Rate Task (PRT) and on the Nine-Hole Pegboard Test (9HPT) as a measure of dysarthria and upper limb dysfunction.
Methods: We defined the time spent on phonation or on hand movement during neuropsychological testing as Verbal Effort Fraction (VEF) and Motor Effort Fraction (MEF). Both were measured experimentally on 65 healthy controls on timed neuropsychological tests (Attentional Matrices, Trail Making Test, Symbol Digit Modalities Test, Verbal Fluencies). We developed correction formulas to normalize VEF and MEF considering the patient's PRT/9HPT, their normality limits, and the test timing. We tested the method on 24 patients with Friedreich Ataxia (FRDA), as a model of motor and speech impairment.
Results: In healthy controls, VEF and MEF ranged between 13.5% and 61.7% of total test time. In FRDA patients, the effect of normalization improved all test results (range: 0.51-48.4%; $p<0.001$). FRDA patients had worst scores in all tests when compared to controls, and the difference remained significant after correction except for the Attentional Matrices. At the individual level, the normalization method improved equivalent scores with fever patients showing impaired scores after correction.
Conclusions: We propose an innovative normalization method to reduce the impact of neurological disability on timed neuropsychological tests. This could be easily integrated in a clinical setting, as it requires a simple preliminary test with the PRT and 9HPT.

150. Reduced cerebral white-matter integrity in Friedreich ataxia is associated with diminution in myelin integrity: The IMAGE-FRDA study

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Introduction:
In Friedreich ataxia (FRDA), white-matter deficits have been observed in the cerebellum, brain stem, cerebrum, and spinal cord, indicating widespread abnormalities in anatomical brain connectivity. However, the underlying pathophysiology of these abnormalities remains unclear (e.g. axonal vs. myelin-related deficits). Understanding white-matter pathology in FRDA has important implications for therapy and biomarker development. This study aimed to characterise white-matter integrity in a large FRDA cohort, and to investigate the extent to which abnormalities may be related to loss of axonal integrity and/or myelin integrity.
Methods:
Thirty-six individuals with genetically-confirmed FRDA and 36 age- and gender-matched healthy control individuals undertook brain Magnetic Resonance Imaging. Diffusion-tensor and magnetisation-transfer imaging protocols were used to undertake whole-brain between- group comparisons of 1) overall white-matter integrity (fractional anisotropy), 2) myelin integrity (radial diffusivity; magnetic-transfer ratio), and 3) axonal integrity (axial diffusivity). Overall white-matter integrity was correlated against the measures of myelin and axonal integrity. Furthermore, each measure was correlated against disease severity, determined by the Friedreich Ataxia Rating Scale (FARS).
Results:
Individuals with FRDA showed significant diffuse deficits in measures of overall white- matter integrity, myelin integrity, and axonal integrity compared with controls, primarily within the cerebellum, brainstem, peri-thalamic regions, corpus callosum and corticospinal tracts. Reductions in overall cerebral white-matter integrity, particularly within the corpus callosum and peri-thalamic regions, were significantly associated with reduced myelin integrity in the FRDA group. Greater FARS scores in the FRDA group were associated with significantly greater abnormalities across all white-matter measures, predominantly within cerebellar and perithalamic regions.
Conclusions:
FRDA is associated with cerebellar and cerebral white-matter abnormalities, which preferentially impact cerebello-thalamo-cortical and cortico-spinal pathways. Furthermore, cerebral white-matter deficits may be particularly driven by myelin damage, indicating a potential treatment target. Measures of white-matter integrity are related to measures of disease severity in FRDA, and therefore warrant further study as disease biomarkers.

151. Sleep and fatigue in Friedreich's Ataxia

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Introduction:
In addition to neurologic symptoms, Friedreich's Ataxia (FA) patients experience poorly characterized systemic symptoms such as impaired sleep, obstructive sleep apnea (OSA), restless legs syndrome (RLS), and fatigue.

Methods:
We recruited subjects from the FA Research Alliance registry with a self-reported diagnosis of FA who resided in the United States to participate in an online survey. Subjects provided demographics and disease state information and completed the Pittsburgh Sleep Quality Index, the Fatigue Severity Scale, and the Visual Analog Fatigue Scale (VAFS).

Results:
Of 171 participants, 42% were male, average onset of symptoms was 17.9y (range 4-51), disease duration was 20.3y (range 2-61), and current age was 38.3y (range 9-77). Average sleep latency was
31.6 minutes (range 2-180) and average sleep duration was 7.4 hours (range 2-12). OSA was endorsed by 16.4%, RLS by 29.8%, and any sleep disorder by 19.9%. Only 25.7% had a sleep study and 20.5% had seen a sleep provider. Presence of OSA correlated with male gender, increased age, disease duration, and FA functional stage. RLS did not correlate with these factors. Average VAFS score was 4.71 (0=worst global fatigue, 10=normal). Presence of OSA, RLS, or other sleep disorders did not predict VAFS score. Smaller GAA repeat expansion correlated with older age of onset and better functional capacity but also with a higher risk of OSA and other sleep disorders.

Conclusions:
Patients with FA experience RLS and OSA with greater frequency than the general population and report significant levels of fatigue. Expected demographic factors correlated with a higher risk of OSA but not RLS. GAA repeats did not correlate as expected with a higher risk of sleep disorders within this small sub- population that was younger, less disabled by FA, and less affected by sleep disorders than the overall study population. Insomnia was not a major issue.

153. A novel oculomotor biomarker in Friedreich’s Ataxia

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Introduction: To investigate the vestibulo-cerebellar interaction in Friedreich's ataxia (FA) to further elucidate the neuro-otological manifestations of this disease and elucidate a possible bio-marker for FA clinical treatment trials.
Background: Friedreich's ataxia (FA) is the most commonly occurring inherited ataxia, and involves widespread neurodegenerative sequelae. Whilst oculomotor, vestibular and cerebellar affects have been documented, little is understood about the clinical consequences of pathology affecting these interacting systems. Impairment of the visually enhanced vestibulo-ocular reflex (VVOR; also called the "doll's head", "doll's eye" or oculo- cephalic reflex) reveals a compound deficit in the three compensatory reflexes involved in eye movement, namely the vestibulo-ocular reflex, smooth pursuit, and the optokinetic reflex.
Materials and methods: A prospective observational study.
Results: We report 20 patients with genetically confirmed FA and uniformly reduced VVOR gain on rapid video-oculography, that is, eye velocity which failed to match head velocity, resulting in gaze position errors, which were corrected with bursts of saccades and perceptible as the clinical sign of an impaired VVOR.
Conclusions: This study further elucidates the pathophysiology of the neuro-otological manifestations of FA. Given the robust and uniform nature of these results, the VVOR is a biomarker planned for implementation in FA treatment trials.

154. Tracking progression in Friedreich's Ataxia (FRDA) to establish biomarkers for clinical trials.

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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 rat- ing scales, echocardiography and one study included MRI of iron deposition in the dentate nucleus of the cerebellum, but none have demonstrated statistically significant improvement despite pa- tients reporting subjective benefits (Perlman, 2012). This has led the scientific community to in- vestigate novel trial designs and explore the identification of new biomarkers that could more reli- ably capture progression of disease.

Methods: This study aims to investigate new ways of measuring disease progression in Friedreich's Ataxia. Innovative and quantitative MRI measures in the brain and spinal cord of up to 24 patients and 6 control subjects will be analysed alongside high resolution imaging of the retina using opti- cal coherence tomography (OCT) and visual acuity checks performed on up to 70 patients. The procedures will be carried out across 3 time points, spanning 22 months. This will be the largest study of its kind to date and should assist in clinical trial development.

Results: We will have collected and analysed data from the first time point and will be able to re- port on the following:

Comparison of controls vs FRDA patients using our novel MRI measures. Confirm/negate findings of previous studies looking at OCT data in FRDA.

Novel methodology for analysing frataxin levels in peripheral blood mononuclear cells.

Conclusions: Correlations between the new measures obtained in this study will be sought. We will also seek to discover if there are any relationships between these new data and the following: age of onset, GAA repeat sequence length, SARA score and Activites of Daily Living Score.

155. Swallowing function declines over 12 months in Friedreich ataxia

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Background: Dysphagia is common in Friedreich ataxia (FRDA). It can lead to aspiration pneumonia and results in reduced quality of life. It is characterised by tongue dysfunction, reduced pharyngeal clearance, delayed pharyngeal swallow, and aspiration. Dysphagia is associated with disease duration and severity, however there are no natural history studies of swallowing in FRDA.
Methods: Twenty-three individuals with FRDA and dysphagia (confirmed via videofluoroscopy, VFSS) were assessed twice 12 months apart. The assessment battery included VFSS, a standardised oral-motor assessment (Frenchay Dysarthria Assessment-2, FDA-2) and a quality of life questionnaire (SWAL-QOL).
Results: Data from the VFSS revealed a significant decline in tongue function, pharyngeal clearance and cricopharyngeal function on solid food. However, severity of penetration/aspiration did not increase. Swallowing-related quality of life and oral-motor function remained stable over the 12 month period.
Conclusions: A decline in function was observed at three anatomical sites important for safe and effective swallowing (tongue, pharyngeal, cricopharyngeal). However, these deficits did not appear to translate into any meaningful difference to the patient and their swallowing related health. The fluctuating nature and general progression of FRDA, level of patients' dysphagia-awareness at follow-up assessment, and psychometric limitations of the assessment tools may have impacted on the results of this study.

THERAPEUTICS AND CLINICAL TRIALS

158. Highly specific ubiquitin-competing molecules promote frataxin accumulation in Friedreich ataxia iPSC-derived neuronal cells.

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Introduction: Friedreich ataxia (FRDA) is mainly caused by reduced expression of frataxin, therefore our therapeutic approach aims at increasing frataxin levels in patients’ cells. Since we have previously shown that frataxin levels are controlled by the ubiquitin-proteasome system, our therapeutic strategy is based on the possibility to increase frataxin levels by preventing its degradation. We have previously characterized a set of small molecules that promote frataxin accumulation by docking on its ubiquitination site thus protecting frataxin from degradation. These compounds are called ubiquitin-competing molecules (UCMs) (Rufini et al., 2011; Rufini et al., 2015). Methods: In order to increase the potency and the efficacy of the identified compounds, we have performed iterative cycles of lead optimization, based on in silico studies, synthesis and in cell validation. Moreover, the effect of the compounds in elevating frataxin levels was evaluated in lymphoblastoid cell lines from FRDA patients, primary FRDA patients’ fibroblasts and in FRDA iPSC- derived neuronal cells.
Results: We have now identified a set of new molecules that show improved efficacy in promoting frataxin accumulation in several lymphoblastoid cell lines and in primary fibroblasts derived from FRDA patients. In particular, these compounds are effective at 1 μM, a concentration 10 times lower that the one described for the previously identified compounds (Rufini et al., 2015). Noteworthy, one of this compound, UCM166, promotes frataxin accumulation also in FRDA iPSC-derived neuronal cells.
Conclusion: These data strongly support the therapeutic potential of this class of compounds and encourage the further development of this therapeutic approach.

Rufini A, Cavallo F, Condo I, Fortuni S, De Martino G, Incani O, Di Venere A, Benini M, Massaro DS, Arcuri G, Serio D, 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.

159. FDA-approved drug screening for Friedreich Ataxia: FDA11 promotes frataxin accumulation at near- physiological levels in FRDA patient-derived cells.

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Introduction: Friedreich Ataxia (FRDA) is an autosomal recessive cerebellar ataxia caused by mutation of the FXN gene, resulting in decreased frataxin expression, mitochondrial dysfunction and oxidative stress. In this work we present one of the possible therapeutic approaches, using a drug repositioning strategy, to find compounds that increase frataxin protein levels.
Methods: To identify drugs able to increase frataxin levels we performed a high-throughput screening of a library containing 853 FDA-approved drugs in HEK-293. Through the use of a transfected frataxin-reporter system that allows a chemiluminescent measure of frataxin levels, we focused our efforts on drugs that function at a post-transcriptional level. Nineteen potential candidate drugs were isolated from the screening. The identified compounds were individually validated and tested for their ability to increase frataxin levels both in HEK-293 cells and in cells derived from patients.
Results: Among the candidates, we focused our attention on compound FDA11. This drug, while showing no significant toxic effects on cell viability, has a substantial impact on frataxin levels. Indeed, FDA11 promotes frataxin accumulation in several FRDA lymphoblastoid cell lines and in primary fibroblasts derived from FRDA patients. Moreover, frataxin accumulation in treated patients cell lines is comparable to frataxin levels in unaffected carrier sibling cells, suggesting that this accumulation could be significant for the purpose of restoring the physiological conditions. Currently, we are testing the efficacy of FDA11 treatment on patients iPSCs-derived neurons.
Conclusions: The evidence presented indicates that FDA11 promotes frataxin accumulation at near-physiological levels in FRDA patient-derived cells, suggesting that this drug could be an interesting candidate for pre-clinical studies as a therapy for FRDA.

160. TAT-MTScs-FXN protects frataxin-deficient neurons and is targeted, processed and functional in mice models of Friedreich ataxia.

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Introduction: One of the approaches to treat Friedreich Ataxia aims to restore frataxin (FXN) function based on frataxin replacement therapies. One of these strategies is based on using TAT-MTScs-FXN, a construct consisting of mature form of frataxin fused to TAT, a peptide enabling membranes crossing and the mitochondrial target sequence from citrate synthase (MTScs) enabling to penetrate the mitochondria (1). In a previous publication, we reported that frataxin-deficient dorsal root ganglia neurons showed altered calcium homeostasis, neurite degeneration and apoptotic cell death (2)

Methods: In this work, we used frataxin-depleted neurons obtained from dorsal root ganglia (DRG), one of the most affected tissues, as cell model of the disease. Reduction of frataxin in DRGs was achieved by transduction with lentivirus containing shRNA silencing sequences. Using this model, we have analyzed the effect of TAT-MTScs-FXN at 1, 3 and 7 mg/mL on decreasing neurodegeneration markers and survival. Mice models of the disease have been also used to test the ability of the fusion protein to reach muscle tissue and its effect on lifespan.

Results: The results show that, treatment with TAT-MTScs-FXN increased cell survival, decreased neurite degeneration and reduced α-fodrin cleavage, an indicator of apoptotic cell death. Also, we show that HSP60, a molecular chaperone targeted to mitochondria, suffered an impaired processing in frataxin-deficient neurons that was relieved by TAT-MTScs-FXN addition. In mice models of the disease, TAT-MTScs-FXN was able to penetrate muscle mitochondria, restore the activity of the mitochondrial succinate dehydrogenase and significantly increase lifespan.

Conclusion: These results support the use of TAT-MTScs-FXN as a treatment for Friedreich Ataxia.

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References:

(1) Marcus D. et al. 2016. Int J Biochem Cell Biol. 81(Pt A):48-56
(2) Mincheva-Tasheva S. et al. 2014. Hum Mol Genet. 23:1829-41

161. Physiotherapy management of the ataxias towards best clinical practice: 2016 guideline update

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Introduction: The principal aim of this systematic review of the literature was to update the physiotherapy section of the Ataxia Management Guidelines (Ataxia UK). The aim of this report is to review the evidence for physical therapy based intervention studies for people with ataxia. PROSPERO registration 2013:CRD42013004323.

Methods: A literature search (v2: 2008-2013) replicated a previous review of: 7 databases (CINAHL, PsycINFO, PubMed Central, British Nursing Index, AMED, EMBASE, SCOPUS), the Web
of Knowledge and the Cochrane Data Base of Systematic Reviews with hand-searching of references (v1: 1980-2009). Search-terms (physiotherapy or physical therapy and ataxia; rehabilitation or exercise or training and ataxia). Criteria included intervention studies (including case studies), opinion pieces or reviews (primarily about ataxia and the role or efficacy of physiotherapy). Scoring of methodological quality was based on the quantitative review form produced by Law et al (1998). Reviewers scored papers independently and final agreed scores achieved through consensus. Three independent physiotherapists developed clinical practice guidance based on their review of the identified papers and clinical experience.

Results: Twenty studies were eligible to add to those original 40 highlighted in the v1 search. One systematic review and 24 research papers were identified: These ranged from randomised controlled trials (n=2) to single case studies (n=8). Methodological quality scores ranged from 4 to 11 (of a maximum 16). Participants had wide-ranging cerebellar pathology.

Conclusions: Dynamic task practice both challenging stability and aiming to reduce upper limb weight bearing seems an important intervention to improve gait and balance. Strength and flexibility training may be indicated in conjunction with these interventions. Whilst there is now modest evidence to support the effectiveness of physiotherapy, insufficient evidence remains to support the efficacy of any one specific intervention. Consistent adoption of valid and reliable outcome measures for this population would improve methodological rigor and interpretation of research.

163. Clinical Trial Readiness for Friedreich's Ataxia Gene Therapy

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Introduction: The overall goal of our Friedreich's Ataxia program is to correct frataxin deficiency in the heart and central nervous system (CNS) by delivery of AAV9 human frataxin (FXN).

Method: We assessed the efficacy of intravenous (IV) delivery of rAAV9-CBA-FXN in a novel conditional knock-down mouse model to prevent and correct the cardiac and neurological disease phenotype. We also compared the efficiency of IV delivery with the combined IV and intrathecal (IT) delivery of rAAV9-CBA-FXN in non-human primates. In parallel, we have been conducting a single-site longitudinal study to identify additional sensitive outcome measures evaluating the cardiac, metabolic and neuromuscular function in individuals with FA in preparation of the current clinical trial. Results: Preliminary data from our preclinical proof of concepts studies showed that: 1) intravenous (IV) injection of AAV9-CBA-hFXN prevents cardiac abnormalities, weight loss and death in the frataxin knock-down model; the combination of IV and intrathecal (IT) AAV9-CBA-hFXN injections in NHPs is safe and leads to human frataxin detection in cerebellum, DRGs and heart tissues. Studies evaluating the effect of IT injections alone in the knock-down model are still ongoing. For the clinical longitudinal study, we enrolled 20 FA subjects and 10 controls. The preliminary data suggests that FA subjects have reduced exercise tolerance during maximal exercise testing as shown by reduced VO2Max. Further, VO2Max correlates well with both the GAA repeat length and the Friedreich's Ataxia Rating Scale (FARS). Conclusion: Based on our observations and ongoing studies, we are planning to conduct GLP toxicology and biodistribution studies in NHP and rodents in support of an IND submission to the FDA. In addition, we are also planning to work with the EU regulatory agencies for a multicenter study.

164. Friedreich's ataxia patients and mice have less mitochondria, and the EMA and FDA-approved drug dimethyl fumarate raises frataxin in cells and mice, and mitochondrial number in mice and humans

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Background and Specific Objectives. The pathophysiological mechanism of Friedreich's Ataxia (FA) is initiated by the deficiency of the mitochondrial protein frataxin, and we studied the consequences of frataxin deficiency on mitochondrial biogenesis in cells, KIKO mice and blood from Friedreich's patients. In addition, we after screening 1600 drugs that went through clinical trials, we studied the mechanism by which Dimethyl Fumarate (DMF) protects Friedreich's cells from death, and demonstrate that DMF increases mitochondrial biogenesis in cells, mice and in human MS patients dosed in vivo, and also dose-dependently increases frataxin in cells.

Methods. Clinical methods were used to dose MS patients with the DMF drug. QRTPCR, QPCR, animal drug dosing, cell culture and siRNA-mediated gene knockdown were used to demonstrate the dependence of mitochondrial biogenesis on frataxin, and the dependence of the mitobiogenic effect of DMF on the Nrf2 target.

Results. We observed deficient mitochondria in FA patient cells, which is dependent on repeat number and frataxin level. Transient knockdown of frataxin in cells produced a defect in mitobiogenesis, that could be rescued by transfection of frataxin. Furthermore we show that human FA patients have a significant defect in mitochondrial biogenesis in blood lymphocytes, and this could be used as an FA biomarker. With DMF, we observe a dose-dependent increase in mitochondrial biogenesis in fibroblasts in the 10 micromolar range, which is the same range that fumarates reach in human plasma after 480mg DMF dosing. In cells at these concentrations there is a consequent increase in mitochondrial biogenesis by Seahorse. Dosing mice in vivo 2 weeks with DMF produces about a 40% increase in mitochondrial biogenesis. DMF has two known targets, i.e. the Keap1/Nrf2 complex, and the HCAR2/Niacin/Betahydroxybutyrate receptor. siRNA-mediated knockdown of these demonstrated that the Nrf2 target is more important for DMF's mitobiogenic effect. DMF also dose- dependently increases frataxin expression.

Conclusions. The mitobiogenesis results demonstrate that Friedreich's and frataxin deficiency cause a specific defect in mitochondrial biogenesis, in FA cells, KIKO mice, and in human FA patients, that could be part of the pathophysiological neurodegeneration mechanism. Secondly since mitochondrial biogenesis is dependent on frataxin level, and taking of blood is considered a less-invasive practice, our results suggest that mitochondrial copy number in blood (or tissues) could now be used as a clinical biomarker of FA disease severity, and could be used in clinical trials of FA's therapeutic development.

The DMF results demonstrate that DMF drug dosed in cells, mice and humans increase mitochondrial biogenesis.

This is the first FDA&EMA clinically-approved drug that has been demonstrated to increase mitochondrial biogenesis. Increasing mitochondrial biogenesis is a general therapeutic strategy in mitochondrial disease, and given the defect in mitochondrial biogenesis in FA observed above, is now a specific therapeutic strategy in FA. We also demonstrate that DMF dosedependently
increases frataxin in human FA patient cells and mice at the same doses it is approved for use in humans. Because DMF has passed through extensive safety testing already, and because it increases frataxin whose deficiency is the only cause of FA, and because it reverses the mitochondrial biogenesis that occurs in Friedreich's, we believe that it should be considered for therapy in Friedreich's ataxia. Some of these results have been

peerreviewed
and are in press, others are in preparation for submission, please consider this as a late-breaking abstract.
Frataxin Deficiency Impairs Mitochondrial Biogenesis in Cells, Mice and Humans.
Jasoliya MJ, McMackin MZ, Henderson CK, Perlman SL, Cortopassi GA. Hum Mol Genet. 2017 Apr 21. doi: 10.1093/hmg/ddx141. [Epub ahead of print]
Dimethyl Fumarate Mediates Nrf2-dependent Mitochondrial Biogenesis in Mice and Humans.Hayashi G, Jasoliya M, Sacc. F, Pane C, Filla A, Marsili A, Puorro G, Lanzillo R, Brescia Morra V, Cortopassi G. Hum Mol Genet. 2017 Apr 28. doi: 10.1093/hmg/ddx167. [Epub ahead of print]

166. Calcitriol, the active form of Vitamin D, reduces apoptotic markers in a neuron model of Friedreich ataxia

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Introduction: Previous data published by our group showed that cultured frataxin-deficient dorsal root ganglia neurons show neurite degeneration, apoptotic cell death and alterations in calcium homeostasis. Frataxin depletion caused activation of CREB to its phosphorylated form and fodrin cleavage by calpain and caspase3, which are markers of apoptotic process (1). Several evidences suggested as that vitamin D could be able to protect frataxin-deficient DRGs from neurodegeneration because anti-apoptotic and neuroprotective effects of the active form of Vitamin D (1α,25(OH)2D3 or calcitriol), have been observed in several neuropathological conditions (2). Also, the last step in the synthesis of the active vitamin D form depends on CYP27B1, a mitochondrial enzyme that hydroxylates 25OHD3 (or calcidiol) to 1α,25(OH)2D3. This enzyme has been localized in neurons and glial cells (3) and is induced when levels of 1α,25(OH)2D3 are low meaning that calcitriol acts as a repressor (4).

Methods: We tested the effect calcitriol in frataxin-deficient dorsal root ganglia neurons. 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 when cultures of frataxin-depleted neurons were treated with doses of 10 and 20 nanomolar, markers of apoptotic cell death such fodrin cleavage or neurite degeneration were clearly reduced. Additionally, a marked increase in CYP27B1 levels observed in frataxin-deficient cultures -thus suggesting low levels of 1α,25(OH)2D3- were reverted to normal values.

Conclusion: These results open an easy therapeutic approach to be considered for patients with Friedreich Ataxia.

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References

(1) Mincheva-Tasheva S. et al. 2014. Hum Mol Genet. 23: 1829-41
(2) Berridge M. J. 2015. Biochem Biophys Res Commun. 460: 53-71
(3) Eyles DW et al. 2005. J. Chem. Neuroanat. 29: 21-30.
(4) Turunen MM., et al. 2007. Nucleic Acids Res. 35: 2734–2747

167. Long-term treatment with thiamine in Friedreich ataxia

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INTRODUCTION. Thiamine (vitamin B1) is a cofactor of fundamental enzymes for the energetic cellular metabolism. Previous studies reported low thiamine levels in the cerebrospinal fluid and pyruvate- dehydrogenase dysfunction in the cells of patients with Friedreich ataxia (FRDA). FRDA is an autosomal recessive disease caused by mutations in FXN gene, which encodes a protein named frataxin that is extremely reduced but qualitatively normal. We investigated in an open trial the effect of long-term treatment with thiamine on the neurological symptoms and the variation of blood FXN mRNA levels in FRDA.
METHODS. Thirty-four FRDA patients were administered with intramuscular thiamine 100 mg twice a week for a long period (meanÅ}sd, 402Å}257 days). Mean age was 36.3Å}11.1 years, mean
age of onset was 17.1Å}9.9 years. Basal levels of plasma thiamine were normal. All patients were evaluated with the Scale for the Assessment and Rating of Ataxia (SARA) at baseline and every three months. Thirteen patients performed echocardiogram before and during treatment, after 450Å}276 days from baseline. FXN mRNA levels were measured in the blood of six patients with quantitative Real Time RT-PCR at baseline and after 12 months of treatment.
RESULTS. Total SARA score improved from 26.6Å}7.7 to 21.5Å}6.2 (p=0.019). Moreover, we detected deep tendon reflexes in 57% of patients with areflexia at baseline, and swallowing improved in 63% of patients with dysphagia. At the echocardiogram, interventricular septum thickness reduced significantly (from 9.54Å}1.76 to 8.85Å}2.00 mm; p=0.016). FXN mRNA blood levels were modestly increased in 50% of patients. CONCLUSIONS. Long-term and continuous thiamine administration was safe and effective in ameliorating neurological symptomatology and echocardiographic parameters in our series of FRDA patients. This improvement was stable over time in all patients, even after three years of treatment. Further studies are required to verify the thiamine role on FXN regulation and to confirm our results.

169. Patient Reported Outcomes in Friedreich's Ataxia after withdrawal from Treatment with ldebenone

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Introduction
Fredreich's ataxia is the most common inherited ataxia, and pathogenesis is known to involve mitochondrial oxidative stress. ldebenone as a potent antioxidant, which has already been evaluated in several clinical trials in FRDA. h inconclusive results. For the first time in an FRDA population, we have employed a treatment withdrawal design to assess whether patients could correctly assess their blinded allocation on either placebo or idebenone continuation.
Methods
Patients taking idebenone for at least 12 months as part of the open-label MICONOS Extension Study were randomised to receive either placebo or idebenone continuation for two-month treatment cycles. The primary endpoint was patient assessment of treatment assignment, and secondary endpoints included early study withdrawal, clinical performance measures and ataxia rating scales. This trial is registered with ClinicalTrials.gov number NCT01303406.
Results
A total of 29 patients were screened and randomised in the study, forming the idebenone group (n=16) and the placebo group (n=13). No significant differences were detected between the idebenone and placebo groups on assessment of treatment assignment or early study withdrawal. A small but significant difference in ataxia rating scale scores was detected between treatment groups when considering ambulatory patients only. Speech intelligibility showed a significant difference between treatments, in favour of the idebenone group.
Conclusions
This study provides no data to suggest that FRDA patients can correctly determine their treatment assignment (idebenone or placebo) over a 2 month period. Future studies with larger cohorts and longer treatment durations should include comprehensive speech assessments and consider sample stratification based on ambulatory status.

170. Morpholino directed alternative splicing of mismatch repair protein mMLH3 in an FRDA mouse model

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Dozens of ataxias and other neurodegenerative disorders are caused by DNA repeat expansion. There is no treatment or cure for any DNA repeat expansion disease. We are studying the mechanism underlying the GAA•TTC repeat expansion that causes Friedreich ataxia (FRDA). Our hypothesis is that selective somatic expansion of GAA•TTC repeats in disease relevant tissues contributes to disease progression. The FRDA mouse model (Tg(FXN)YG22Pook) exhibits region specific GAA•TTC repeat expansion chiefly in the CNS and most markedly in the cerebellum, consistent with this hypothesis. Our work in human cells indicates that somatic expansion of GAA•TTC repeats requires three sequential steps involving: 1) transcription through the DNA repeat 2) MutSβ (MSH2/MSH3 heterodimer) and 3) MutLγ (MLH1/MLH3 heterodimer). MLH3 is expressed in humans as two isoforms. MLH3 isoform 1 includes a conserved endonuclease domain, while MLH3 isoform 2 lacks this cutting domain. We have shown that MLH3 isoform 2 does not promote repeat expansion in human cells. Skipping the exon encoding the endonuclease domain in both mice and men retains the MLH3 reading frame and effectively shifts MLH3 to isoform 2. We targeted the mouse MLH3 gene with two types of morpholino splice-switching oligonucleotides (SSOs), which were well tolerated. In some tissues, such as kidney, we can reliably alter mMLH3 splicing via tail-vein injection. In the CNS we had to resort to intracerebroventricular (ICV) administration via osmotic pump to effect splice switching in the cerebellum. Although at the time of submission we have yet to sustain mMLH3 isoform switching in the cerebellum sufficiently long to change the GAA•TTC repeat expansion rate we remain optimistic given the recent success of intrathecal delivery of splice switching reagents such as nusinersen for spinal muscular atrophy in humans.

171. RT001 First-in human Clinical Trial Demonstrates Safety, Favorable Pharmacokinetics, and Early Signals of Efficacy in Friedreich's Ataxia

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Introduction: RT001 is a deuterated linoleic acid ester that inhibits lipid peroxidation, reducing cellular damage and recovering mitochondrial function in degenerative diseases such as Friedreich's Ataxia (FRDA). A first-in-human study was conducted to evaluate the safety, pharmacokinetics, and preliminary efficacy of RT001 in FRDA patients.
Methods: We conducted a double-blind, comparator controlled trial with 2 doses in FRDA patients. Subjects were randomized 6:3 to receive either RT001 (1.8 g/d or 9.0g/day), or a matching dose of linoleic acid ester as comparator for 28 days. Patients were counseled to observe a low polyunsaturated fatty acid (PUFA) diet (3-5 g/day) throughout the study. The primary endpoints were safety, tolerability and pharmacokinetics. Secondary endpoints included the Friedreich's Ataxia Rating Scale (FARS)-NEURO, timed 25-foot walk (T25FW) with electronic motion sensing, and cardio-pulmonary exercise testing (CPET).
Results: 19 patients enrolled in the trial, and 18 completed all study measurements (12 on active drug and 6 on comparator), median age 35 years, median baseline FARS NEURO = 58. RT001 was found to be safe and tolerable, with plasma levels approaching saturation by 28 days. Patients recorded food intake in a diary and were compliant with diet restrictions. Deuterated arachidonic acid (a brain penetrant metabolite of linoleic acid) was present in plasma. One patient with low BMI experienced steatorrhea taking high dose RT001. There was an improvement in peak workload in the drug group compared to placebo ($p = 0.02$), and a strong improvement trend in peak V02, gait in the T25FW-1 measured by electronic sensors, and FARS- NEURO.
Conclusions: RT001was found to be safe and tolerable over 28 days, and appeared to improve multiple clinical measures in this study. Longer-term evaluation of RT001 in FRDA is warranted.

172. Stimulating neural repair through bone marrow stem cell fusion in models of ataxia

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Introduction

Neuronal cell loss is a critical feature in both genetic and acquired ataxic conditions. Strategies to attenuate neuronal cell injury are crucial to reducing the clinical burden of ataxia and accumulation of neurological disability. A major conceptual consideration in central nervous system repair is how damaged neurons, given their enormous complexity, can possibly be replaced or restored. The phenomenon of cell fusion between infiltrating, healthy bone marrow-derived cells and injured neural cells offers a tantalising solution; it may also provide an opportunity to introduce therapeutic ‘donor’ genetic material to boost cell survival.

Methods

Using both in vitro and in vivo experimental techniques and analysing human brain tissue, we have explored in-depth the process of cell fusion in animal models of ataxia and in patients with cerebellar disease.

Results

In animal models of ataxia and human post-mortem brain tissue, we provide evidence of disease-related increases in neuronal cell fusion and heterokaryon formation. We show that in genetic and acquired models of ataxia, post fusion, genes derived from the donated bone marrow-derived cell nucleus are expressed within the host neuronal cell, demonstrating proof of concept of a potential gene therapy within the nervous system. Moreover, we show that fusion between bone marrow-derived cells and existing neuronal cells leads to the formation of electrically active neurons, restoring their function.

Conclusion

Our studies have provided novel and fundamental insights into the ways in which nerve cells can be protected and their survival prolonged. Given this potential solution to repairing neurons in adult life, harnessing fusion as a potential gene therapy and/or neuro-regenerative treatment could be clinically valuable to a wide range of patients with otherwise untreatable neurological diseases.

173. Evaluation of AMX0035, a novel combination therapy for the treatment of neurodegenerative diseases, in cellular models of Friedreich's Ataxia

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Introduction: Amylyx Pharmaceuticals has developed a novel therapeutic, AMX0035, for the treatment of neurodegenerative diseases. AMX0035 is a combination of two small molecules, Sodium Phenylbutyrate (PB) and Tauroursodeoxycholic Acid (TUDCA), designed to block neuronal death and neuroinflammation through simultaneous inhibition of ER and mitochondrial stress.

Amylyx discovered a synergy between these two compounds when administered in combination across multiple preclinical models. The combination showed a synergistic increase in neuronal viability in an H2O2-mediated oxidative insult model. Additionally, AMX0035 has been evaluated in in vivo models of ALS and Alzheimer's disease (AD) and shown to have neurobiological effect and strong blood-brain barrier permeability.

Furthermore, AMX0035 was evaluated in preliminary in vitro studies in Friedreich ataxia patient derived fibroblasts and isolated sensory neurons derived from the KIKO FA mouse model.

Methods and Results: FA patient fibroblasts were grown in culture media containing betahydroxybutyrate,
a selection media for mitochondrial dysfunction that has been shown to
cause primary FRDA fibroblasts to grow poorly and lose viability over several days.1 Treatment with AMX0035 improved cell viability relative to control under BHB-culture conditions.

KIKO-derived sensory neurons develop a relevant pathogenic phenotype for FA: the fragmentation of mitochondria and reduced mitochondrial mass. AMX0035 treatment at the combination dose of 50uM TUDCA + 500uM PB, was effective in reversing mitochondria fragmentation and loss of mitochondrial mass.

Conclusion: These studies demonstrate that AMX0035 promotes cell viability, including protecting frataxin-deficient cells from metabolic stresses, potentially through a mitochondrialtargeted
mechanism of action, and may have application as a therapeutic for FA. Additional evaluation of the molecular mechanisms by which AMX0035 protects frataxin-deficient cells are underway in vitro and in vivo models of mitochondrial dysfunction. Further, AMX0035 will enter clinical trials in ALS and AD in mid-2017

1 Cotticelli, M. Grazia, et al. "Phenotypic screening for Friedreich ataxia using random shRNA selection." Journal of biomolecular screening 20.9 (2015): 1084-1090.

175. Rationale and Trial Design of a Study of the Efficacy and Safety of Omaveloxolone in Patients with Friedreich's Ataxia (MOXIe)

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INTRODUCTION:

Omaveloxolone, an Nrf2 activator and NF-kB suppressor, targets dysfunctional inflammatory, metabolic, and bioenergetic pathways. Efficacy data for omaveloxolone from Part 1 of a Phase 2 study in Friedreich's ataxia (MOXIe, NCT02255435) showed statistically significant improvement in neurological function assessed using the modified Friedreich's ataxia rating scale (mFARS), with a subgroup of patients without musculoskeletal foot deformities (pes cavus) having greater improvement. A randomized, placebo-controlled, double-blind portion of the trial (MOXIe), which could support registration, has been designed to assess the efficacy and safety of omaveloxolone in Friedreich's ataxia patients.

METHODS:

Approximately 100 Friedreich's ataxia patients will be randomized 1:1 to either omaveloxolone 150 mg or placebo to be administered once daily for 24 weeks. Patients with pes cavus will comprise no more than 20% of patients enrolled. Randomization will be stratified by pes cavus status. An independent data safety monitoring board will monitor the study.

RESULTS:

The primary endpoint is change from baseline in mFARS score relative to placebo at Week 24. Secondary endpoints are the change from baseline in peak work during maximal exercise testing relative to placebo at Week 24, and the proportion of patients "much improved" or "very much improved" relative to placebo in the Patient Global Impression of Change and Clinical Global Impression of Change scales. Exploratory efficacy endpoints will include the proportion of patients at Week 24 with mFARS improvements at or better than specified cutoff values (e.g., -2, -3, etc), change in FA Activities of Daily Living score, SF-36, change in performance on a 9- hole peg test, change in performance on a 25-foot timed walk test and frequency of falls.

CONCLUSIONS:

The Part 2 MOXIe trial design is a robust, registrational, placebo-controlled study that includes evaluation of a variety of clinically relevant endpoints; many of these endpoints correlate with FA disease progression.

178. Effect of Diazoxide on Friedreich ataxia models

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Friedreich ataxia (FRDA) is an inherited recessive disorder caused by a deficiency in the mitochondrial protein frataxin. In this study, we tested diazoxide, a drug commonly used as vasodilator in the treatment of acute hypertension, on cellular and animal models of FRDA. Diazoxide is able to increase frataxin protein levels in FRDA lymphoblastoid cell lines, via the mTOR pathway. Moreover, prolonged oral administration of 3mpk/d diazoxide in frataxindeficient transgenic YG8sR mice was found to be safe, but produced variable effects concerning efficacy. YG8sR mice showed improved beam walk coordination abilities and footprint stride patterns, but a generally reduced locomotor activity. Moreover, they showed significantly increased frataxin expression, improved aconitase activity and decreased protein oxidation in cerebellum and brain mitochondrial tissue extracts. Further studies are needed before this drug should be considered for FRDA clinical trials.

180. Rehabilitation improves health and well-being in individuals with Friedreich ataxia.

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Introduction: Progressive mobility decline, upper limb ataxia, muscle weakness and spasticity have a significant impact on the ability to perform activities of daily living and the quality of life for individuals with Friedreich ataxia (FRDA). Primary treatment of FRDA is based on symptom management and maintenance of function, which includes rehabilitation. We aimed to compare the effectiveness of a six-week rehabilitation program in individuals with FRDA to no therapy. The effects of a home exercise program (HEP) following rehabilitation were also examined. Methods: We conducted a single-blinded randomised controlled trial. Nineteen participants with FRDA were randomised to an immediate rehabilitation group or a six-week delayed-start control group. Rehabilitation involved outpatient land and aquatic physiotherapy. This was followed by a six-week HEP. The primary outcome was the Functional Independence Measure (FIM). Secondary outcome measures included the motor domain of the FIM (m-FIM), Friedreich Ataxia Impact Scale (FAIS), Berg Balance Scale (BBS) and Friedreich
Ataxia Rating Scale (FARS). Outcomes were administered at baseline and six weeks. Additionally, response to the HEP was measured for all participants. Results: There was a significant within-group increase in the m-FIM for the immediate group ($t(9)=2.41$, $p=0.039$) indicating that individuals undergoing rehabilitation made functional gains. Participants in the immediate group had an average 12.4% improvement in health and well-being at six weeks, compared to a 3.5% worsening in the control group ($t(17)=3.40$, $p=0.003$), as indicated by the FAIS body movement subscale. Collated data from both groups (n=18) identified a reduction in the FARS ($p=0.016$), and an increase in the BBS for non-ambulant participants ($p=0.026$) after the HEP, both indicating improvement. Conclusions: This study found that rehabilitation improves function as well as the health and wellbeing of individuals with FRDA, providing compelling evidence that short-term rehabilitation should be offered to individuals with FRDA.

182. Clinical trials in Friedreich ataxia: pre-clinical evidence of efficacy is essential

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Introduction

When a new treatment is tested in humans, safety is the primary concern of regulatory agencies and Ethics Committees (or IRBs). They impose appropriate pre-clinical assessment of potential toxicity and strict rules for first-in-human trials, with safety monitoring remaining an essential requirement throughout all phases of therapeutic development. However, as pointed out in a recent Comment in the journal Nature (Kimmelman J and Federico C, Nature 2017; 542:25-27), robust pre-clinical evidence of efficacy is equally essential to justify testing an experimental therapeutic in people.

Methods

Results of past clinical trials in Friedreich ataxia (FRDA), published in peer-reviewed journals or disclosed in press releases, have been reviewed along with the pre-clinical evidence of efficacy that supported the trial.

Criteria considered for any proposed therapeutic for FRDA include: 1) target a biological process with a major pathogenic role, as supported by robust, consistent and reproducible evidence; 2) interfere with such process, partially or entirely interrupting the pathogenic cascade as early as possible; 3) correct key phenotypic abnormalities in cellular and animal models of the disease; 4) have appropriate pharmacological properties, such as lack of offtarget effects, favorable pharmacokinetics (PK), good blood-brain barrier (BBB) penetration.

Results

Clinical trials in Friedreich ataxia (FRDA) have so far failed to identify any effective treatment, leaving a major unmet medical need. While flaws in the way trials have been designed and conducted are often cited as a major factor, I would argue that lack of appropriate evidence of efficacy, obtained in well conducted pre-clinical studies, has been largely responsible of these repeated failures.

A few examples can illustrate these points.

Trials of antioxidants in FRDA have so far been disappointing. Looking back at the preclinical evidence for their use, it is often flagrantly insufficient. Idebenone was never tested in appropriate models systems before moving to human trials. A study in a cardiac mouse model only followed several human trials, with only modestly positive results despite the very large doses utilized (Seznec H et al. HMG 2004; 13:1017-1024). Even evidence that antioxidants target a relevant pathogenic mechanism is questionable. While oxidative stress does occur in several model systems (e.g. Codazzi et al., HMG 2016; 25:4847-4855), it does not appear to be needed for neurodegeneration induced by frataxin deficiency (e.g. Seznec H et al., HMG 2005; 14:463-474, and more recently Chen et al. eLife 2016; 5:e20732), suggesting that antioxidants are not likely to interrupt the pathogenic cascade at an early step. While it can be argued that these are relatively benign drugs, it must be carefully assessed if they may be worth testing when the expected benefit is likely to be limited.

Although frataxin restoration appears to be the best possible therapeutic approach for FRDA, several compounds supposed to upregulate frataxin expression, such as erythropoietin (EPO) or gamma interferon (IFNG), failed in clinical trials. Again, they had limited preclinical evidence of efficacy. Frataxin-inducing properties were usually modest. Model systems did not closely reproduce the human disease, so evidence of correction of a relevant phenotype was lacking. Furthermore, no mechanism of action was identified.

Considerations about PK properties, BBB penetration, and receptor distribution should also have induced caution.

Conclusion

Even if it always provides some useful information, a failed trial has a very high human and

financial cost. It breaks hopes and wastes resources. Furthermore, analysis of didease progression in large patient cohorts demonstrated that proof of efficacy in FRDA requires long trials with a substantial number of participants, limiting the number of studies that can be performed at any given time. We must therefore avoid committing patients and time, the most precious resources, to test treatments whose potential efficacy is not supported by strong enough experimental evidence.

183. CAT-4001 improves mitochondrial function in a Friedreich's ataxia model

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The frataxin deficiency that underlies Friedreich's ataxia (FA) leads to oxidative stress and decreased mitochondrial function, which may play significant roles in disease pathology. Impaired nuclear translocation of Nrf2 may be the causative factor for oxidative neuronal damage in FA. CAT-4001 is a novel, CNS-penetrant small molecule conjugate of monomethyl fumarate (MMF), which activates Nrf2, and the omega-3 fatty acid, docosahexaenoic acid (DHA), which inhibits NF-κB, coupled via a linker designed to be enzymatically cleaved, enabling simultaneous intracellular release of the active components with concomitant synergistic pharmacology.

Degeneration of large sensory neurons is a hallmark of FA, and associated defects can be observed in dorsal root ganglion (DRG) derived neurons from frataxin-deficient mice (KIKO). KIKO DRG neurons show mitochondrial abnormalities in terms of mitochondrial fragmentation and altered bioenergetics. Treatment of DRG neurons from normal (WTWT) or KIKO mice with CAT-4001 increased expression of the Nrf2-target gene, Hmox1, indicating pharmacological modulation of the target pathway. To assess CAT-4001 effects on mitochondrial abnormalities, cultures were treated with CAT-4001 and mitochondrial lengths were measured using fluorescence microscopy. Compared to WTWT neurons, KIKO neurons showed a decreased mitochondria length within their axons, consistent with mitochondrial fragmentation. However, the axonal mitochondria in CAT-4001 treated KIKO neurons were longer, and of comparable lengths to the mitochondria in WTWT neurons, suggesting that CAT-4001 could prevent mitochondrial fragmentation.

We evaluated mitochondrial respiration in a mouse muscle cell-line (C2C12). Treatment with a pro-oxidant stressor leads to markedly decreased oxygen consumption in these cells. CAT-4001 reversed the H2O2-mediated decreases in oxygen consumption in a concentration-dependent manner. Combined with the effects in reversing mitochondrial fragmentation seen in the KIKO neurons, these results suggest that CAT-4001 is likely to improve mitochondrial bioenergetics. In conclusion, CAT-4001 may represent an effective approach to improve mitochondrial function for the treatment of FA.

184. Modification of Frataxin with BBB-shuttles to increase brain access

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Introduction

The development of an efficient protein replacement therapy for Friedeich ataxia (FA) is hampered by the presence of the Blood-Brain barrier (BBB). The natural protection of the brain is also the main obstacle when delivering therapeutics. BBB-peptide shuttles are compounds able to circumvent the BBB increasing the transport of substances linked to them. We propose the modification of expressed Frataxin (FXN) with various protease resistant BBB-shuttles in order to improve its BBB penetration. BBB cell-based models are used to test and compare the constructs prepared.

Methods

Mature FXN with and without a selected mitochondrial localization signal (FXN81-210 and MLS-FXN81-210) were expressed in E. Coli. Modification with the BBB-shuttles of choice was carried out by site-selective modification strategies or by modifying the most reactive solvent exposed residues. All the constructs were conveniently characterized. BBB-cell based models, both endocytosis and transcytosis, were used to stablish the BBB transport capabilities of the different constructs.

Results

When challenging a human BBB cell-based model with the designed FXN construscts differences in transport were observed when comparing number, type and location of the BBBshuttles.

Endocytosis experiments allowed for intracellular distribution of the constructs.

Those that reached the mitochondria on selected cells lines and had good transport were selected for further experiments.

Conclusions

Modification of FXN with protease resistant BBB-shuttles is an attractive approach to develop an efficient protein replacement therapy since the newly designed FXN derivatives have better BBB permeability in cell-based in vitro models of the BBB.

185. Biophysical Characterization of the Recombinant Human Frataxin Precursor

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Introduction: One outstanding strategy to increase the concentration of active frataxin (FXN) inside the mitochondria is the production of recombinant variants of FXN with the capability to cross cell membranes using a TAT-derived peptide fused to FXN precursor [1-4]. For an eventual TAT-FXN-based therapy, the integrity of protein conformation is essential. Not enough information is available about the conformation of precursor FXN1-210. Here, we investigated the conformation and stability of a recombinant precursor (TAT-His6-FXN1-210), which includes a TAT peptide in the N-terminal region plus a histidine tag.

Methods: His6-TAT-FXN1-210 was expressed in Escherichia coli codon plus ROSETTA2pLysDE3
cells and purified to ≥ 95% (SDS-PAGE). Mass and aggregation state were evaluated by ESI-MS and MALS/DLS. Circular dichroism and fluorescence measurements were performed. GdmCland temperature-induced unfolding experiments were carried out. Cysteine desulfurase NFS1/ISD11 activation by FXN was investigated (methylene blue method). Protein transduction was studied using FITC-labeling TAT-His6-FXN1-210. Immune response against TAT-His6-FXN1-
210 (0.05 or 0.25 nmol) was measured using C57 mice. Specific antibodies to FXN variants were tested by ELISA.

Results: We optimized expression and purification conditions that maintain the protein soluble, even after freezing and thawing, free of aggregation, oxidation or degradation. His6-TAT-FXN1-210 is monomeric, with the N-terminal stretch (residues 1-89) mostly unstructured, and the C-terminal domain folded. GdmCl-induced unfolding of the precursor is reversible, cooperative and the protein is stable. Temperature-induced unfolding/aggregation was not reversible but the addition of low concentrations of GdmCl makes it reversible. His6-TAT-FXN1-210 activates desulfurase in vitro. Precursor was translocated across cell membranes and our results support the notion that the C- terminal fragment is not immunogenic at these concentrations.

Conclusions: His6-TAT-FXN1-210 exhibits an enhanced propensity to aggregate. Conditions were set that keep the protein stable and soluble after freeze/thaw, with minimal autoproteolysis. Low GdmCl concentration prevents N- terminal-mediated aggregation.

1. Marcus, D., et al., Heterologous mitochondrial targeting sequences can deliver functional proteins into mitochondria Therapeutic approaches for the treatment of Friedreich's ataxia. Int J Biochem Cell Biol, 2016. 81(Pt A): p. 48-56.
2. Vyas, P.M., et al., A TAT-frataxin fusion protein increases lifespan and cardiac function in a conditional Friedreich's ataxia mouse model. Hum Mol Genet, 2012. 21(6): p. 1230-47.
3. Kim, M.J., et al., Tat-Frataxin protects dopaminergic neuronal cells against MPTPinduced toxicity in a mouse model of Parkinson's disease. Biochimie, 2012. 94(11): p. 2448-56.
4. Strawser, C.J., K.A. Schadt, and D.R. Lynch, Therapeutic approaches for the treatment of Friedreich's ataxia. Expert Rev Neurother, 2014. 14(8): p. 949-57.

187. DAO inhibitor preclinical therapeutic studies for FRDA

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Friedreich ataxia (FRDA) is an inherited progressive neurodegenerative disorder caused by a trinucleotide (GAA) repeat hyper-expansion within intron 1 of the of the frataxin gene, FXN, which instigates transcriptional deficiency. The neurological defect in FRDA is primarily caused by the degeneration of dorsal root ganglia and cerebellar neurons and degeneration of axons in peripheral nerves, dorsal roots, and posterior columns, depriving the cerebellum of sensory input to coordinate movement.

It is speculated that high levels of D-amino acid oxidase (DAO) enzyme activity affects regular neural transmission extensively in the cerebellum, by degrading D- serine and in turn inducing stereotyped behavior and ataxia. In this study, a small molecule inhibitor of DAO, TAK-831, was investigated using FRDA (YG8sR) mice. Two chronic dosing experiments were performed by administering either vehicle or 3mpk TAK-831 by daily gavage for 14 days on groups of ten FRDA mice at approximately 4 months of age and again at 9 months of age, with a 97 day interval between experiments. Age and sex matched vehicle-treated WT mice were also used as controls.

Results indicated no significant FRDA-like disease or TAK-831 effects for the 4 month old mice. However, a progressive FRDA-like disease effect was seen for the 9 month-old vehicle-treated FRDA mice versus WT mice, whilst the TAK-831-treated FRDA mice showed significantly improved motor coordination ability compared with vehicle-treated FRDA mice. Moreover, the dose of TAK-831 used was well tolerated, with no effect on mouse weight and qRT-PCR results showed no effect of TAK-831 on FXN gene expression. The outcomes of this study encourage the continued study of TAK-831 as a potential therapy for FRDA; however more research needs to be carried out to understand the effects of this DAO inhibitor therapeutic compound.

188. HMTase inhibitor preclinical therapeutic studies for FRDA

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Friedreich ataxia (FRDA) is an inherited progressive neurodegenerative disorder caused by an intronic GAA trinucleotide repeat expansion within the frataxin gene (FXN), which encodes a mitochondrial protein, frataxin. This abnormal GAA expansion plays a role in histone modification, subjecting the FXN gene to heterochromatin silencing. Therefore, inducing a more relaxed chromatin structure at the FXN gene by inhibiting various histone markers may have beneficial therapeutic outcome.

Recent studies have shown that histone H3 lysine 9 trimethylation (H3K9me3) and histone H3 lysine 27 trimethylation (H3K27me3) are enriched in the flanking regions of expanded GAA repeats, where acetylation marks are correspondingly reduced.

Here we have studied two histone methyltransferase (HMTase) inhibitor compounds, BIX01294 and GSK-126, to specifically target and reduce H3K9me3 and H3K27me3 levels, respectively, in FRDA human and mouse model (Y47R, YG8R, YG8sR and YG8LR) fibroblast cells,

using concentrations ranging from 1nM to 10μM.

Potential cell toxicity for each drug was assessed using the PrestoBlue cell viability assay, followed by collection of cells for biochemical and molecular analysis. Thus far, we have detected lack of cell toxicity and a consistent 1.6-fold increases in FXN mRNA and frataxin protein levels when using BIX-01294 at concentration from 1nM to 1uM. In contrast, GSK126 induced inconsistent changes of FXN mRNA and frataxin protein levels in different cell types at non-toxic concentration, including decreases in FXN expression. For further analysis, we are currently investigating the levels of methylated histone marks at the FXN locus after BIX01294 or GSK-126 treatment by performing chromatin immuno-precipitation (ChIP). Additionally, we are combining BIX01294 and GSK-126 together to detect any potential synergistic effect on FXN gene expression.

189. BBB-shuttle decorated DNA nanocarriers to treat Friedreich's ataxia.

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Introduction

Brain delivery is one of the major challenges in drug development, as the blood–brain barrier (BBB) prevents most drugs from reaching their central nervous system targets. BBB-shuttle peptides with their capacity to cross the BBB and transport cargoes of distinct sizes and types that can not cross unaided – offer great promise to safely overcome this formidable obstacle.1-3

In the case of Friedreich's ataxia (FA), we envisaged to go significantly beyond the state-of-theart in gene delivery by combining the properties of two well-known DNA nanocarriers (such as viral vectors and PLGA nanoparticles) with the targeting properties of our peptides that function as BBB-shuttles.

Methods

We have chemically decorated these two DNA nanocarriers (viral and non-viral) with synthetic protease-resistant BBB-shuttle peptides. Also, we have incorporated some cell penetrating peptides (CPPs) at the surface of the PLGA NPs to help on their internalization at the target cells.

We have characterized the physicochemical (Z-potential, size and encapsulation efficiency) and gene delivery properties of the obtained nanoconstructs, including in vitro and in vivo evaluation of the transfection of the encapsulated DNA, as well as, transport evaluation using BBB cell-based models. All these tools have been used to optimize the set of nanoconstructs prepared and the most promising and robust have been selected to be further developed and studied.

Results and Conclusions

Based on our obtained results, we have decided to focus our future efforts in the transport of synthetic BBB-shuttle decorated PLGA nanoparticles. These systems are highly advantageous as delivery vehicles since they are simple to prepare, scale-up and can be decorated in a more controlled and reproducible manner. In addition, they generally have less pro-inflammatory effects than viral particles. Although some optimization is still required, our nanoparticles are not only able to encapsulate the cDNA encoding for Frataxin but also the complete Frataxin gene.

References

1.- B. Oller-Salvia, M. S.nchez-Navarro, E. Giralt, M. Teixid., Chem. Soc. Rev., 2016, 45, 4690-4707.
2.- R. Prades, B. Oller-Salvia, S.M. Schwarzmaier, J. Selva, M. Moros, M. Balbi, V. Graz., J.M. de La Fuente, G. Egea, N. Plesnila, M. Teixid., E. Giralt, Angew. Chem. Int. Ed., 2015, 54, 3967-3972.
3.- B. Oller-Salvia, M. S.nchez-Navarro, S. Ciudad, M. Guiu, P. Arranz-Gibert, C. Garcia, R.R. Gomis, R. Cecchelli, J. Garc.a, E. Giralt, M. Teixid., Angew. Chem. Int. Ed., 2016, 55, 572- 575.

191. Combining multiple therapeutic strategies for Friedreich's ataxia (FRDA): antioxidant metallic nanoclusters as coadjutants for gene and stem cell therapy.

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Introduction FRDA pathology is caused by guanine–adenine–adenine trinucleotide repeat expansion within the first intron of frataxin (FTX) gene, leading to epigenetic silencing. Since mitochondrial FTX controls cellular iron use and redox status maintenance, its lack causes an increased level of reactive oxygen species. We report an effective lentivirus FXN gene delivery to FTX deficient mesenchymal stem cells (MSCs), inducing improvement of neurological functionalities when transplanted in vivo. We also identify antioxidant nanoclusters (NCs) able to block ROS–dependent apoptotic pathways in the FRDA pathology. Methods MSCs from FRDA patient bone marrows were transduced with a lentiviral vector for FXN expression. After LV transduced MSC characterization by FACS, IF, and WB analyses, engineered cells were systemically injected in Fxntm1MknTg (FXN)YG8Pook/2J mice. Behaviour tests (rotarod and treadmill) were performed. Brain tissues were harvested for IF staining and WB. FRDA MSCs labelled with AuAg NCs were characterized to evaluate mitochondrial ROS scavenger activity. Results After LV transduction, MSCs showed the preservation of mesenchymal marker expression (CD73, CD44, CD90 and CD105), colony forming abilities, and capacity to differentiate into multilineages. Comparison with untreated animals revealed i) in vivo FTX rescue; ii) increased number of cerebellar cells expressing Tuj1 neuronal marker; and iii) improvement trend of motor skills in mice injected with engineered MSCs. In addiction, AuAg NCs entered the mitochondria of FRDA MSCs where they reduce ROS levels lowering cell sensitivity to oxidative stress. Conclusions The results confirm the gene and stem cells-based therapeutic applicability to treat the neuronal degeneration in FRDA. The suitability of metallic NCs as anti oxidant agents represents a crucial point as implemental strategy for further ameliorating the progressive ROS mediated degeneration. Indeed, a combined approach based on NCs nasal inhalation in autologous transplanted FRDA patients may be considered as a step further into a clinically relevant treatment.

192. Increased frataxin expression induced in Friedreich ataxia cells by new TALEs fused with a transcription activation domain.

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Introduction: The FXN gene expression is reduced in Friedreich ataxia (FRDA) patients due to an increase in the number of GAA trinucleotides in intron 1. The frataxin protein, coded by that gene, plays an important role in the iron metabolism in the mitochondria. TALE (proteins targeting the regulatory region of the FXN gene, fused with transcription factors (TF) were used to increase the expression of that gene.
Methods: Thirty one (31) different TALEs targeting 14 sequences of the FXN gene were produced.
Results: The best 3 TALEs increased FXN gene expression by up to 19-folds in different FRDA fibroblasts. An AAV9 virus was used to deliver the selected TALE-TF genes to the YG8R mouse model to validate the efficacy of these effectors in vivo.
Conclusion: The results show that these selected TALE-FTs induced the transcriptional activity of the endogenous FXN gene as well as the expression of the frataxin protein in vitro and in vivo in the heart and the skeletal muscles. The higher frataxin expression increased the aconitase activity, which is reversibly modulated by the frataxin level in mitochondria.

193. In vivo deletion of the GAA repeats from the intron 1 of the human frataxin gene using the CRISPR system delivered with PHP.B-serotyped AAV in the YG8R mouse model.

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Introduction: The CRISPR system has been proved to efficiently modify DNA molecules both in vitro and in vivo. The system allows to specifically target genome sequences with minimal offtarget effects. The actual CRISPR technology toolbox is constantly growing and many different nucleases are available for a broad range of gene editing and modification purposes.
Methods: In order to remove the GAA repeat expansion, a major mutation of the FXN gene known to be involved in the Friedreich Ataxia, we designed sgRNA and used either S. pyogenes or S. aureus Cas9 nuclease to cut in intronic regions flanking the repeat. In vitro experiments have been done using mouse YG8sR isolated fibroblasts containing a human FXN mutated transgene while in vivo experiments were performed on YG8sR mice injected with adenoassociated viruses (AAV).
Results: In vitro results showed a 2-fold increase of the frataxin protein expression following correction of the mutation in YG8sR fibroblasts. Therefore, the aconitase activity in treated cells was restored to wild-type level. In vivo results in YG8sR mice showed efficient delivery of a PHP.B-serotyped AAV encoding CRISPR components in tissues of the central nervous system, including the cerebellum and the dorsal root ganglia. The deletion of the GAA repeat expansion (200 repeats) was detected using digital droplet PCR (ddPCR) and percentages of edited molecules varied between the analyzed tissues, with a range between 0.2 and 2.1% after one-month injection. Long- term experimentations are ongoing and will be further discussed.
Conclusion: The CRISPR system can be used to remove the GAA repeat expansion from a mutated FXN gene and could represent a one-time treatment for the Friedreich Ataxia, a monogenic disease for which there is no cure.

194. Combining transcranial direct current stimulation and intensive physiotherapy in patients with Friedreich's Ataxia: a pilot study.

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Friedreich's ataxia (FRDA) is a neurodegenerative disorder affecting primarily the dorsal columns of the spinal cord and cerebellum. FRDA leads to progressive disability and reduced lifespan. No cure is currently available. The effect of physiotherapy in FRDA has been debated with no definitive recommendation either in favor or against it. The transcranial Direct Current Stimulation (tDCS) is a safe and non-invasive technique applying small-intensity currents directly to the scalp leading to cortical excitability. tDCS has been applied to various neurological conditions, including ataxias, with promising results. However, the use of tDCS to potentiate the effects of rehabilitative- intervention has never been tested. We propose a pilot randomized double-blind study in FRDA undergoing intensive-physiotherapy treatment and randomly allocated into active- vs sham-tDCS. We recruited 4 patients: 3F/1M; aged 20.25Å}3.86 years; disease duration 10.75Å}3.77 years; GAA1 662.5Å}159.87, Friedreich's Ataxia
Rating Scale (FARS) staging 1-4 and no contraindication to tDCS. The intensive-physiotherapy program consisted in 5-weeks of 2 sessions/day targeting balance and core-stability. The anodal-stimulation was applied over M1 and cerebellar cortex bilaterally once/day for 2-weeks, 20 minutes, 2mA intensity in the active-tDCS. Patients were assessed at pre-/posttreatment with FARS, 6-minute walking test (6-MWT), MiniBEST, BERG. Descriptive analysis was performed to compare the differences between pre- and post-treatment results in all patients and each group separately.
All patients tolerated the treatments protocol and showed post-treatment functional improvement in all the scales applied. Active-tDCS showed a greater improvement in the FARStot score, upright- stability section and in 6MWT when compared to sham-tDCS group. Intensive-physiotherapy in FRDA ambulatory patients results in measurable improvements in functional scales. The effect on walking and upright-stability is potentiated by tDCS to M1 and cerebellum. This difference might be due to the effect of the cortical stimulation in pathways involved in the motor control. Larger RTC are needed to confirm these results.

195. Speech Rehabilitation in Friedreich ataxia

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Introduction: The loss of the ability to speak is a devastating and inevitable outcome of many neurodegenerative diseases. It results in daily disadvantage, stigmatisation, social marginalisation and underemployment. Disordered speech is an inevitable consequence of hereditary ataxias.
Methods: We have designed a home-based, intensive four-week speech exercise programme designed to improve speech in people with hereditary ataxia. The treatment protocol is based on principles of motor learning and neuroplasticity with a focus on improving speech intelligibility and vocal control. Exercises and feedback were created to enhance selfmonitoring and include computer based aural, visual and results feedback and selfmanagement.
17 patients with a degenerative ataxia (7x ARSACS, 5x SCA6, 2 x SCA1, 2 x SCA3) have completed the study so far. Efficacy was measured by an expert listener blinded to timepoint rating intelligibility of speech samples from the study participants using Direct Magnitude Estimation. (DME). DME dictates that samples are compared to a pre-identified anchor.
Results: In these 17 participants, mean (SD) intelligibility was 98.0 (29.8) points at baseline and 119.5 (25.4) 4-weeks post-baseline with Pearson's correlation coefficient of 0.9. The relative (%) increase in speech intelligibility from baseline to post-treatment was statistically significant (geometric mean of the post-treatment/baseline ratio 1.25, 95% CI [1.16, 1.35], $p<0.0001$) using a two-sided paired t-test on base-2 log-transformed outcome data. Relative improvements from baseline in intelligibility of 5-10% represent clinically meaningful change in participants' speech including significant change in voice quality and naturalness in a variety of tasks (eg. conversation). We saw individual relative increases in intelligibility between -7-80% in our pilot data. On an individual level, 16/17 (94%) patients responded to treatment.
Conclusions: Our preliminary speech rehabilitation data has yielded promising results. Our pilot data shows that even mild dysarthria improves with our intervention. The relative improvement is larger for those ataxia participants (SCA) with more severe dysarthria. A larger trial continues.

196. EPI-743 (Alpha-tocotrienol Quinone) Demonstrates Long-Term Improvement in Neurological Function and Disease Progression in Friedreich's Ataxia

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Study Supported by: Edison Pharmaceuticals, Mountainview, CA, provided all study support and investigational product.
Introduction: FRDA is an inherited ataxia caused by impairment of mitochondrial iron-sulfurcluster protein assembly. EPI-743 is a compound that targets oxidoreductase enzymes essential for redox control of metabolism. The objective of this study was to evaluate the longterm safety and clinical effects of EPI-743 in Friedreich's Ataxia (FRDA).
Methods: We conducted a multicenter trial that included a 6-month multiple dose placebo controlled phase, followed by an 18-month phase in which all subject received treatment with EPI-743. The primary study objective was low contrast visual acuity as assessed by Early Treatment Diabetic Retinopathy Study (ETDRS). The key secondary study endpoint was neurological function as assessed by the Friedreich’s Ataxia Rating Scale (FARS) – NEURO.
Results: A total of 63 subjects were enrolled in the trial; 61 completed the initial phase. EPI-743 was found to be safe and well tolerated, and demonstrated consistent pharmacology. During the placebo- controlled phase, there were no significant improvements in the primary or secondary endpoints using raw scores. However, significantly more patients taking low-dose EPI-743 had a 3-point or greater improvement (equivalent to one year of symptom progression in natural history cohorts) in the FARS NEURO than patients taking placebo ($p = 0.047$). Longitudinal modeling at 24 months revealed significantly improved disease progression in all drug groups when compared to natural history data. Using FA Clinical Outcome Measure data, the mean FARS increase (worsening) in untreated patients over 24 months is 4.8 points, compared to a mean decrease (improvement) in all patients taking EPI-743 of 1.8 points (2-tailed t-test with equal variance $p=0.00001$).
Conclusions: EPI-743 was safe and well tolerated, and although it did not reach the primary endpoint following six months, long-term treatment resulted in significantly improved neurological outcome after 24 months compared to natural history data.

197. RNA therapeutics for Friedreich's Ataxia

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Friedreich's ataxia (FRDA) is a fatal untreatable neurodegenerative disease caused by expansions of guanine-adenine-adenine (GAA) repeats in intron 1 of the frataxin (FXN) gene. Homozygous GAA repeat expansion leads to significantly decreased quantities of frataxin mRNA and protein. In principle, any molecular manipulation eliciting an increase in frataxin levels should be beneficial. Recent studies of mammalian transcriptomes have identified new classes of small and long non-coding regulatory RNAs that may be used as therapeutics. Here we describe the application of recently discovered classes of small RNA molecules, RNAa, and long non-coding RNAs, SINEUP, to respectively increase transcription and translation of frataxin and rescue the disease phenotype in FRDA cellular models.

We show that four independent artificial miRNAs targeting FXN promoter (FXN- RNAa) are able to elicit a moderate, however reliable, upregulation of frataxin mRNA in HEK293T cells. The transcriptional upregulation activity of the best-performing FXN-RNAa was further validated in immortalized peripheral lymphocytes, obtained from FRDA patients, and in patients' fibroblasts.

In a parallel approach, upon screening in HEK 293T cells, we selected a number of FXN-specific SINEUPs that increase frataxin protein quantities acting at a post- transcriptional level. SINEUPFXN
activity is retained when tested in human neuroblastoma cells. Most importantly, SINEUPFXNs could elicit a 2-fold increase in frataxin protein quantities in fibroblasts derived from FRDA patients.

Our results pave the way for new therapeutic strategies to cure FRDA and provide new classes of RNAs for molecular medicine.

199. What to look for in a clinical trial? How clinical trials can be interpreted differently in reviews.

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INTRODUCTION:

Before changing your practice in the light of a recently published clinical trial or review, it is important to assess if the clinical trial or review is relevant, valid and appropriate for the population you are treating. In particular, did the author just use reviews from people known to him or did the author do a systematic search? Is it an objective review?

Every year, researchers and scientists publish more than three million new articles in scientific journals. It has been estimated that a health professional would need to read 20 articles every day just to stay on top of their field. These articles range from background information, expert opinion, case series, case controlled studies, cohort studies, randomised controlled trials, critically appraised individual articles, critically appraised topics to systematic reviews at the top of the mountain. A systematic review gives a thorough yet abridged view of the evidence in a particular field.

OBJECTIVES:

1) To explore different types of clinical trials and reviews
2) To critical appraise clinical trials which are used in reviews
3) To look at the framework for systematic reviews

RESULTS:

This poster aims to:
1) summarise what to look for in a clinical trial,
2) give a framework to assess reviews,
3) explain the theory of pooling the results of small studies to form a meta-analysis,
4) help resolve contradictory findings among different studies on the same question.

Example of quantitative summary known as meta-analysis from systematic review

DISCUSSION:

Systematic Reviews were conceived as early as the 17th century when astronomers began to see the value of combining data sets instead of choosing between one and the other. Increasingly, medicine required some sort of synthesis, particularly in simplifying the eventual clinical decisions of medical practitioners. Cochrane Systematic Reviews aim to answer a particular research question using a structured approach, explicitly formulated, reproducible, are constantly updated.

Systematic reviews are prepared by a team of at least two reviewers who have a thorough understanding of the clinical area and review methodology to minimize bias and significantly reduce human error.

Keywords: Review, Bias, meta-anaylsis