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# Welcome

We wish you a very warm welcome to the International Ataxia Research Conference 2015 in Windsor. This conference is hosted and organised by four ataxia organisations from four different countries, which we feel is evidence of the international collaboration platform created to advance treatments for these diseases. The partners, Ataxia UK, Ataxia Ireland, GoFAR (Italy) and FARA (US) have been assisted by a Scientific Steering Committee which has put together a robust programme covering diverse and relevant topics in ataxia research.

The conference covers all hereditary and sporadic ataxias. Sessions have deliberately not been themed by the specific types of ataxias but rather across the spectrum of basic, translational and clinical research relevant to advancing us from diagnosis to treatment as we believe there is significant benefit to be gained from understanding advances in the broader field and applying lessons learned. The sessions have been organised to promote sharing based on topic areas or focus/stage of research; genetic research and diagnosis; molecular mechanisms and cellular pathways; animal and cell models; drug discovery and therapeutic approaches; and clinical research and trials The organisers and Steering Committee were especially encouraged by the number of abstract submissions across all themes, most particularly in drug discovery and therapeutic development. We see this as an important milestone for our scientific and patient communities. We are also very pleased to have an interview with a representative of the European Medicines Agency who will be giving advice and information on the regulation of the development of treatments.

People with ataxia are at the heart of everything we do and to highlight this we have invited three people with ataxia to give presentations from their different perspectives. We are also delighted that many patient group representatives are attending the conference and that the annual meeting of euro-Ataxia, (the federation of ataxia charities in Europe) will also be meeting here in parallel to the conference - giving a perfect opportunity for patients, patient groups and researchers to meet, and learn from one another.

We believe this may be the largest ataxia research conference to date, demonstrated in the number of oral and poster presentations, delegates and sponsors! We are grateful to our Committee and session chairs for the time and effort in reviewing abstracts and preparing our program. We are pleased that so many invited speakers and academic researchers are joining us from around the world and that the conference has attracted a significant number of representatives from pharmaceutical companies – this all highlights the relevant science and collaboration fuelling fast growing interest in ataxia research.

An event this large would not be possible without the generous support of our many sponsors to whom we are extremely grateful. Fourteen pharmaceutical companies have kindly provided sponsorship and four ataxia patient groups have partnered with the four organising charities and given their financial support to the conference.

Over the next few days we will take steps together to bring us closer towards the development of much needed treatments for the ataxias. We hope you learn from the research reported and ideas shared; network with other participants; and leave with new colleagues in your research circle, and new inspiration and urgency for your work.

| Sue Millman | Barbara Flynn | Mina Ruggeri | Jen Farmer |
|---|---|---|---|
| Ataxia UK | Ataxia Ireland | GoFAR (Italy) | FARA (US) |

# Organisers

**Conference Planning Committee:**

Ataxia UK (www.ataxia.org.uk)

Ataxia Ireland (www.ataxia.ie)

FARA (www.curefa.org)

GoFAR (www.atassiadifriedreich.it)

**Scientific Steering Committee:**

Paola Giunti, MD, PhD, Principal Clinical Research Associate, Institute of Neurology, UCL, London

Barry Hunt, PhD, Professor, Trustee of Ataxia UK

Michele Lufino, PhD, Research Fellow, Molecular Neurodegeneration and Gene Therapy, University of Oxford, UK

Giovanni Manfredi, MD, PhD, Professor, Brain and Mind Research Institute, Weill Cornell Medical College, New York, USA

Massimo Pandolfo, MD, Professor, Department of Neurology, Hopital Erasme, Universite Libre de Bruxelles, Belgium

Hélène Puccio, PhD, Research Director, Institute of Genetics and Molecular and Cellular Biology, University of Strasbourg, France.

Roberto Testi, MD, Professor, University of Rome Tor Vegata, Italy

Rob Wilson, PhD, MD, Professor, Department of Pathology and Laboratory Medicine, University of Pennsylvania Medical Center, Philadelphia, USA

Jen Farmer, MS, CGC, Executive Director, FARA USA

Julie Greenfield, PhD, Research Projects Manager, Ataxia UK

Julie Vallortigara, PhD, Research Officer, Ataxia UK

Mina Ruggeri, Founder and Director, GoFAR

# Sponsors

The Organisers would like to express their thanks and gratitude to the conference sponsors:

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# Biographies

## Talks from people with ataxia

### Helen Kearney: *My life with ataxia*

At the age of 12, Helen developed back pain. Some months later it was discovered she had severe scoliosis and she had to have surgery. This would mean she would be off horse riding for 6 months which was a significant problem for Helen as a 12 year old girl. The surgery was a 2 stage procedure over a two week period. Helen's family noted that her recovery was slow. She sat around all day which was not usual for her. When she was 2 weeks post operative, she wanted to visit a horse which was 30 km away. Her mum told her she would have to walk around their house before she could visit the horse. She did that and soon went to see the horse. A year later she was diagnosed with Friedreich's ataxia. Her "love affair" with horse continued and culminated by being a triple paralympian medallist in London 2012. Currently she is training her new horse "Rocky" for Rio in 2016.
Helen has a website if you wish to find out more about her: www.helenkearney.ie

### Kyle Bryant: *Life is about how we react*

Kyle Bryant is an athlete, keynote speaker and director of the bicycle ride fundraiser, rideATAXIA as a staff member of the Friedreich's Ataxia Research Alliance (FARA). rideATAXIA currently has 5 locations in the US and has raised over $3 million for FA research since 2007. Despite his diagnosis of FA at the age of 17, Kyle has completed numerous long distance bike rides including "The World's Toughest Bike Race", Race Across America (RAAM) in 2010 as part of 4 man Team FARA.

### Harriet Bonney: *The patient in the chair*

Harriet Bonney was initially diagnosed with Friedreich's ataxia in 1991 but the diagnosis has since been revised to an idiopathic cerebellar ataxia. She grew up in Lancashire and moved to Glasgow in 1995 to study Medicine at University where she graduated with a BSC(Med Sci) and an MBChB. Harriet has been a Friend of Ataxia UK for over 20 years now, a Trustee for the past 6 years and a Chairman for the past two and a half years. She has recently taken early medical retirement from her job as a Speciality Doctor in General Adult Psychiatry, and now lives in Lytham St Annes.

## After Dinner Talk – Wednesday 25th March

### Professor Sophie Scott: *The Neuroscience of Laughter*

Professor Sophie Scott is the Deputy Director of the Institute of Cognitive Neuroscience at University College London. Prof Scott studied psychology at the Polytechnic of Central London followed by a PhD at UCL. She then worked in Cambridge at the MRC Applied Psychology Unit before returning to UCL to the newly established Institute for Cognitive Neuroscience. Prof Scott was awarded a Welcome Trust Fellowship in 2001. Her work addresses the neurobiology of human vocal behaviour and vocal communication from sound to speech.

## Pre Dinner Talk – Friday 27th March

### Avril Daly (EURORDIS – Rare Diseases Europe)

Avril Daly was elected to the EURORDIS Board of Directors in 2009, and has been Vice-President of EURORDIS since 2012. As CEO of the Irish charity Fighting Blindness Avril is responsible for raising awareness of retinal degenerative diseases among the general public, health care professionals and policy makers as well as the implementation of the organisations strategic development plan.

Avril is the current Chairperson of the Genetic and Rare Disorders Organisation (GRDO), the Irish voluntary umbrella group supporting Irish families affected by genetic and rare disorders. In 2011 she was appointed to the steering committee working towards the development of the Irish National Plan for Rare Diseases at the Department of Health.

Avril represents Retina Europe at the European Patients Forum and through her work with Fighting Blindness, is a board member of the Medical Research Charities Group (MRCG and, the Irish Platform for Patients' Organisations, Science and Industry (IPPOSI).

Avril represents EURORDIS at International Conferences throughout Europe and beyond and is part of the interim working group for the European Year for Rare Diseases.

For more information about EURORDIS, please visit their website http://www.eurordis.org/

# Participant information

## Presentations

Lectures will take place in the Hanover Suite.
The poster presentations will be in the Chapel.

**Poster session 1, 2 and 3**
Wednesday 25 March
18:30 – 20:15

**Poster session 4 and 5**
Friday 27 March
12:00 – 14:00

**Poster session 6 and 7**
Friday 27 March
18:00 – 19:30

Each poster has been assigned a number, which appears alongside its abstract in this booklet. Posters presented in session 1, 2 and 3 should be put up during registration on Wednesday 25 March and taken down by the end of the day. Posters presented in session 4 and 5 and session 6 and 7 should be put on Friday 27 March morning and taken down by the end of the day. Please could presenting authors stand by their posters during their poster session, as indicated above.
Velcro for hanging posters will be provided at the registration desk.

Attendees requiring a Certificate of Attendance for the meeting should contact the registration desk or email research@ataxia.org.uk. They will be issued after the event.

## At the Beaumont Estate

The registration fee includes lunches and refreshments for the duration of the conference. The accommodation fee includes breakfast and the evening meal.
Refreshments will be served in the Hanover Lounge.
Breakfast will be served from 06:45 in the Beaumont Estate restaurant.
Any extra expenses at the Beaumont will be charged to you at the end of of the stay.

Set near historic Windsor, Beaumont Estate is a majestic hotel and conference venue. Just 15 minutes from London Heathrow, close to the M3, M4 and M25 with direct train links into London, the hotel combines a world class, state of the art conference and event venue. The parking at the venue is free of charge. To book a taxi, please contact Windsor Cars on 01753677677 or ask at the hotel reception. Beaumont Estate is 5 minutes from Egham, Windsor Central and Windsor and Eton Reviverside railway stations. For more information about trains to travel to London, please visit this website: http://ojp.nationalrail.co.uk/service/planjourney/search

## Social programme

If you have booked for the evening in Oxford, the drinks reception and the dinner will be held on Thursday 26 March at:
The Oxford Town Hall
St Aldate's
Oxford
OX1 1BX

Coaches will leave Beaumont Estate straight after the afternoon session on Thursday. Delegates will have a free time of around two hours in Oxford before the drinks reception.

## Coverage of the conference

**Wi-Fi at the Beaumont:**
WiFi is available across the venue, and is included in the registration fee. Delegates can get a WiFi passcode for the day at the hotel reception or registration desk.

**Tweeting and Blogging at IARC**
We very much want to encourage discussion of this conference via Twitter, Facebook and similar social networks. Please use the conference hashtag: #IARC2015.

However, some speakers may not wish to see their presentations disseminated at this stage and in order to promote maximum discussion and the open exchange of information at the Conference you are asked to **respect any requests from speakers to keep information they give in their presentations confidential** and not to disseminate their research via Twitter or any other route.

Speakers have been made aware of this policy, and asked to inform delegates at the beginning of their presentation if they wish any of the contents to remain confidential.

**Telephones**
Delegates are asked to ensure that their mobiles are on silent whilst lectures are in progress and to refrain from making or answering calls.

**Photography**
Please note that photographs taken at this event may be used for promotional purposes by the Organisers, e.g. by inclusion on their website and/or materials. If you have any concerns or queries regarding this, please visit the registration desk

# Programme overview

## Wednesday 25 March

11:00 – 13:00 Registration and light lunch
13:00 – 13:15 Welcome/ housekeeping
13:15 – 17:45 **Session 1: New genes and developments in diagnosis of the ataxias**
18:30 – 20:15 **Poster sessions 1, 2 and 3** and welcome drinks
20:15 – Dinner and Prof Sophie Scott (UCL) on the neurology of laughter

## Thursday 26 March

8:30 – 12:45 **Session 2: Genetic and molecular mechanisms of the ataxias**
12:45 – 14:00 Lunch
14:00 – 16:00 **Session 3a: Cellular and animal models of Friedreich's ataxia**
14:00 – 15:45 **Session 3b: Cellular and animal models of other ataxias**
16:15 – Coaches leave for Oxford
17:15 – 19:15 Free time
19:15 – 19:45 Drinks reception at Oxford Town Hall
19:45 – Dinner in Oxford Town Hall
22:45 – Coaches leave for Beaumont Estate

## Friday 27 March

8:30 – 12:00 **Session 4: Cellular and systemic pathways**
12:00 – 14:00 Lunch and **poster sessions 4 and 5**
14:00 – 18:00 **Session 5: Drug discovery and emerging therapeutic strategies**
18:00 – 19:30 **Poster sessions 6 and 7**
19:40 – Conference Dinner and pre-dinner talk from Eurordis

## Saturday 28 March

8:30 – 12:45 **Session 6: Biomarkers and functional measures**
12:45 – 14:00 Lunch
14:00 – 17:30 **Session 7: Clinical trials and trial design**
17:30 – 17.40 Close of conference

## Session 1: New genes and developments in diagnosis of the ataxias

**Wednesday 25th March**

*Chairs: Andrea Nemeth (University of Oxford and Oxford NHS Trust, UK) and Michel Koenig (Institut Universitaire de Recherche Clinique, Montpellier, France)*

Invited speakers

13:25 Michel Koenig (Institut Universitaire de Recherche Clinique, Montpellier, France):
*Recessive ataxia by partial loss of function: a common theme*

13:55 Andrea Nemeth (University of Oxford and Oxford NHS Trust, UK):
*The future of diagnostics in ataxias and implications for therapeutics*

Selected presentations

14:25 Marie Coutelier (INSERM, Paris, France):
*GRID2 mutations span from congenital to mild adult onset cerebellar ataxia*

14:45 Marios Hadjivassiliou (Royal Hallamshire Hospital, Sheffield, UK):
*The aetiology of progressive cerebellar ataxia: Prospective evaluation of 1234 patients.*

15:05 Rebekah Jobling (The Hospital For Sick Children, Toronto, Canada):
*PMPCA mutations cause abnormal mitochondrial protein processing in patients with non-progressive cerebellar ataxia*

15:25 Break

15:55 Stefania Magri (Fondazione IRCCS, Istituto Neurologico Carlo Besta, Italy):
*A comprehensive NGS gene panel for the genetic diagnosis of hereditary cerebellar ataxias*

16:15 Rebecca Schule (Hussman Institute for Human Genomics, University of Miami, USA):
*Collaborative gene identification strategies in hereditary ataxias*

16:35 Matthis Synofzik (Hertie-Institute for Clinical Brain Research, Germany):
*Exome and panel sequencing in a large cohort of early-onset ataxias: novel genes and genetic mechanisms*

16:55 Dineke Verbeek (University of Groningen, Netherlands):
*The identification of novel spinocerebellar ataxia disease genes using next generation sequencing approaches*

17:15 Margit Burmeister (University of Michigan, USA):
*Surprising results from next generation sequencing: summary of >50 ataxia exomes sequenced*

17:35-17:45 Close of session

Invited Speaker: Michel Koenig (Institut Universitaire de Recherche Clinique, Montpellier, France)

## Recessive ataxia by partial loss of function: a common theme

Michel Koenig, Claire Guissart

Laboratoire de Génétique Moléculaire et unité INSERM UMR_S 827 IURC (Institut Universitaire de Recherche Clinique), Montpellier Cedex 5, France

Recessive progressive ataxias represent a heterogeneous group of degenerative disease that affect the cerebellum and/or the spinocerebellar and sensory tracts of the spinal cord. The growing list of identified recessive ataxia genes indicate that they encode proteins involved in a variety of distinct pathways (mitochondrial, peroxysomal, cytoskeleton, chaperones, chanels, transporters, RNA metabolism, chanels, transporters, DNA repair, etc …). Rather than an alteration of specific metabolic pathways of the cerebellum and/or spinocerebellar tracts, we proposed that recessive progressive ataxias are the consequence of an exquisite sensitivity of the cerebellar/spinocerebellar neurons to even mild metabolic insults (Anheim et al. NEJM 2012, 366: 46-56). In other words, we propose that recessive progressive ataxias are due to partial loss of function mutations (the case for the vast majority of genes, such as in the metabolic ataxias), or are due to mutations in specific members of redundant gene families (for only 6 genes : ADCK3 in ARCA2, ABHD12 in PHARC, ANO10 in ARCA3, ATM in ataxia-telangiectasia, APTX in AOA1 and SETX in AOA2), or are due to mutations affecting redundant detoxifying pathways (TTPA and MTTP genes in vitamin E deficiencies and chaperones in ARSACS and Marinesco-Sjögren syndrome). I will illustrate the case of ataxias due to partial loss of function mutations from two syndromes that we recently identified, ataxia-epilepsy syndrome (SCAR12) due to WWOX mutations and ataxia-deafness (Lichtenstein-Knorr syndrome) due to SLC9A1 mutation, and from the metabolic ataxia syndromes.

Invited Speaker: Andrea Nemeth (University of Oxford and Oxford NHS Trust, UK)

## The future of diagnostics in ataxias and implications for therapeutics

Molecular diagnostics is undergoing a revolution as a result of new gene sequencing technologies. In the last decade the development of high throughout sequencing has transformed the diagnostic rate for rare ataxias. Two years ago we reported an 18% detection rate with targeted capture of 57 ataxia genes. This targeted capture forms the basis for the Oxford ataxia diagnostic panel, which now includes 91 genes. Detection rates using exome sequencing are even higher, although this is not yet routinely available in clinical practice. The next phase of diagnostic sequencing will be focused on the whole genome and in the UK will include a new initiative known as Genomics England.

High throughput sequencing has also enabled numerous new 'ataxia' genes to be identified. However, even exome or genome sequencing does not find the cause in all cases and the next challenge will be to determine the genetic mechanisms accounting for other cases.

Here I will review the current state of play in the field of molecular diagnostics. I will then go on to consider the impact of improved diagnostics within the research environment. In particular, I will focus on the identification of new genes using high throughput sequencing, how these technologies are likely to develop and how our knowledge of molecular pathways leading to ataxia might impact on the development of novel therapies.

## GRID2 mutations span from congenital to mild adult onset cerebellar ataxia

Marie Coutelier[1, 2, 3, 4, 5, 6], Lydie Burglen[7, 8, 9], Emeline Mundwiller[4], Myriam Abada Bendib[10], Diana Rodriguez[7, 8, 11], Sandra Chantot-Bastaraud[7, 9], Christelle Rougeot[7, 12], Marie Anne Cournelle[13], Mathieu Milh[14], Annick Toutain[15], Delphine Bacq[16], Vincent Meyer[16], Alexandra Afenjar[7, 11], Jean-François Deleuze[16], Alexis Brice[1, 2, 3, 4, 17], Delphine Héron[17], Giovanni Stevanin[1, 2, 3, 4, 6], Alexandra Durr[1, 2, 3, 4, 17]

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**Objectives**: In a large family of Algerian origin, we aimed to identify the genetic mutation segregating with simultaneous presence of adult onset, paucisymptomatic, slowly progressive, cerebellar ataxia in 7 adults and congenital ataxia in one child. Simultaneously, we aimed to assess the involvement of *GRID2* mutations in 144 patients with congenital cerebellar ataxia.

**Methods**: We used a combined approach of linkage analysis and whole exome sequencing in one family; and a targeted gene panel sequencing approach in 144 congenital ataxias.

**Results**: In the large SCA family, we identified a missense mutation (c.1966C>G/p.Leu656Val) in the *GRID2* gene, in a heterozygous state in adults and in a homozygous state in one child with congenital ataxia, compatible with a semidominant transmission pattern. In 144 patients affected with congenital ataxia, we identified two missense de novo *GRID2* mutations in two children (c.1960G>A/ p.Ala654Thr, c.1961C>A/p.Ala654Asp). They affect the same amino acid as the previously described *Lurcher* mutation in mice; the variant in the large family concerns a nearby amino acid.

**Conclusions**: In humans, *GRID2* had only been involved in ataxia through complete loss of function mutations due to exon deletions, with an autosomal recessive pattern of inheritance. We report the first point mutations in this gene, with putative gain of function mechanisms, and a semidominant transmission as was observed in the *Lurcher* mice model. Interestingly, cerebellar ataxia is the core phenotype, but with variable severity ranging from very mild adult onset to congenital onset ataxias linked to both the heterozygous and homozygous state of the variant, and the position of the mutation.

## The aetiology of progressive cerebellar ataxia: Prospective evaluation of 1234 patients

Marios Hadjivassiliou[1], Priya Shanmugarajah[1], Joanne Martindale[2], Diane Friend[1]

*[1] Academic Department of Neurosciences, The Royal Hallamshire Hospital, Sheffield, United Kingdom, [2] Department of Molecular Genetics, Sheffield Childrens HospitalNHS Trust, Sheffield, United Kingdom*

Progressive cerebellar ataxia is a disabling neurological condition of diverse aetiology. It can be genetic or acquired. There is an ever expanding number of genetic causes with over 50 different genes being implicated. Acquired ataxias include both immune and degenerative causes. Making a diagnosis of less common causes of cerebellar ataxia necessitates evaluation at dedicated Ataxia Centres with expertise in the clinical evaluation and investigation of patients with ataxia.

The Sheffield Ataxia Centre was set up 20 years ago and is dedicated to the clinical evaluation of such patients. It receives referrals from all over the UK. During the last 20 years, 1,234 patients with progressive ataxia have been assessed. All patients are offered regular follow up appointments and most patients attend on a regular basis. Out of 1,234 patients 242 (20%) had a family history (familial ataxia) and 992 (80%) did not (sporadic ataxia). Amongst 992 patients with sporadic ataxia 266 (27%) patients had no diagnosis despite extensive investigations (idiopathic sporadic ataxia). Gluten ataxia accounted for 22% and ataxia due to excessive alcohol intake for 14%. A genetic diagnosis was made despite the absence of family history in 13%. The 3 commonest genetic diagnoses in this group were Friedreich's ataxia (FA), SCA6 and episodic ataxia. Other causes of sporadic ataxia included cerebellar variant of multi-system atrophy (MSA-C) in 12%, paraneoplastic cerebellar degeneration in 3%, anti-GAD associated ataxia in 2%, permanent phenytoin induced ataxia in 2%, cerebellitis in 2% and superficial siderosis in 1% . Four percent of patients had myoclonic ataxia and 1% had ataxia with palatal tremor.

Of 242 patients with familial ataxia 170 (70%) had an autosomal dominant and 72 (30%) autosomal recessive family history. Out of the whole group of 242 patients a genetic diagnosis was achieved in 48% (this figure is prior to the introduction of ataxia panel testing using exome sequencing). The commonest genetic ataxias in the AD group were SCA6 and EA2 and in the AR group FA and SPG7.
A history of early onset ataxia was present in only 8% of the total number of 1,234 patients. Of these early onset cases 47% were found to have a genetic cause. Even in sporadic early onset ataxias a genetic cause was identified in 46% by comparison to only 13% in late onset sporadic cases.
In total a potentially genetic cause was identified in 366 (30%) of the whole group of 1,234 patients (242 with family history plus 124 with genetic confirmation from the sporadic group). Despite extensive investigations 22% of patients had no clear aetiology for their ataxia. Additional data on genetic ataxia causes within the familial group using exome sequencing will be available at the time of presentation.

## PMPCA mutations cause abnormal mitochondrial protein processing in patients with non-progressive cerebellar ataxia

Rebekah Jobling[1], Mirna Assoum[2, 3], Oleksandr Gakh[4], Susan Blaser[5], Julian Raiman[1], Cyril Mignot[6], Emmanuel Roze[7, 8, 9, 10, 11], Alexandra Dürr[7, 8, 9, 10, 12], Alexis Brice[7, 8, 9, 10, 12], Chitra Prasad[13], Tara Paton[14], Andrew Paterson[14], Nicole Roslin[14], Christian Marshal[14], Jean-Pierre Desvignes[2, 3], Stephen Scherer[14, 15], Guy Rouleau[16], André Mégarbané[17], Grazia Isaya[4], Valérie Delague[2, 3], Grace Yoon[1, 18]

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Non-progressive cerebellar ataxias are a rare group of disorders which comprise approximately 10% of static infantile encephalopathies. We report here the identification of mutations in PMPCA in 17 patients from 4 families affected with cerebellar ataxia, including the large Lebanese family previously described with autosomal recessive cerebellar ataxia and short stature of Norman type and localized to chromosome 9q34 (OMIM #213200). All patients present with non-progressive cerebellar ataxia, and the majority have intellectual disability of variable severity. PMPCA encodes a-MPP, the alpha subunit of mitochondrial processing peptidase, the primary enzyme responsible for the maturation of the vast majority of nuclear-encoded mitochondrial proteins, which is necessary for life at the cellular level. Analysis of lymphoblastoid cells and fibroblasts from patients homozygous for the PMPCA p.Ala377Thr mutation and carriers demonstrate that the mutation impacts both the level of the alpha subunit encoded by PMPCA and the function of mitochondrial processing peptidase. In particular, this mutation impacts the maturation process of frataxin, the protein which is depleted in Friedreich ataxia. This study represents the first time that defects in PMPCA and mitochondrial processing peptidase have been described in association with a disease phenotype in humans.

## A comprehensive NGS gene panel for the genetic diagnosis of hereditary cerebellar ataxias

Stefania Magri[1], Daniela Di Bella[1], Elisa Sarto[1], Serena Caldarazzo[1], Lorenzo Nanetti[1], Caterina Mariotti[1], Cinzia Gellera[1], Michel Koenig[2], Peter Bauer[3], Franco Taroni[1]

*[1] Unit of Genetics of Neurodegenerative and Metabolic Disease, Fondazione IRCCS Istituto Neurologico Carlo Besta, Italy, [2] Laboratoire de Génétique Moléculaire et Unité INSERM UMR_S 827, IURC, France, [3] Institute of Medical Genetics, University of Tübingen, Germany*

Hereditary ataxias are genetically highly heterogeneous with >50 genes identified thus far. However, >50% of the patients remain undiagnosed. With the advent of NGS, virtually all known disease genes can be tested at once, hugely increasing the expected diagnostic yield. Aim of this study was to analyze with an NGS gene panel all the patients referred to our laboratory and negative for mutations in the more common ataxia genes. We used two different NGS approaches within two different research projects:

1) within the E-Rare EUROSCAR project, 142 ataxic patients referred from different European partners were analyzed using a HaloPlex-based gene panel targeting the coding regions of 127 genes involved in recessive and dominant ataxia. Patient inclusion criteria for analysis were: progressive ataxia, exclusion of nongenetic causes, family history suggestive of autosomal recessive ataxia (AR) or sporadic (S) patients with onset before age 40. We contributed with 34 Italian patients (14AR and 20S) previoulsy tested for FRDA, AVED, or APTX, as appropriate;
2) within an Italian Ministry of Helath project, we developed a TruSeqCustomAmplicon (TSCA, Illumina) exon enrichment assay for 76 genes for both dominant (SCA) and recessive (SCAR) spinocerebellar ataxias. With this strategy, we analyzed 52 patients (9AD, 15AR, 28S) with ataxia.

Approximately 90% of the target regions were sequenced at >20X coverage. A standard bioinformatic pipeline for mapping and annotation yielded a total of 200-400 variants per subject, which could be reduced to <5 with a filtration strategy that included a local ethnically-matched mutation database. Overall, pathogenic mutations were identified in ~20% (17/86) of patients. In particular, we identified mutations in 2 challenging genes, *SYNE1* (SCAR8, 5 index cases) and *SACS* (ARSACS, 2 index cases), which are extremely difficult to be studied by conventional sequencing because of their length. Further recessive ataxias included SCAR9 (*ADCK3*, 2 families) and cerebrotendineous xanthomatosis (*CYP27A1*, 1 index case). In the SCA group, we identified mutations in SCA23 (2 sporadic cases) and SCA19 (3 sporadic cases), and in the very rare genes SCA5 and SCA14 in 2 large multigeneration families. Different missense variants of uncertain pathogenicity were identified in genes responsible for dominant ataxia and several heterozygous mutations were identified in recessive genes. All high-quality (Q30) variants were confirmed by Sanger sequencing indicating reliability of this approach. Further analyses are required for the validation of uncertain variants and the assessment of the presence of large in/del mutations in recessive genes in which heterozygous mutations have been identified. In conclusion, our data confirm the efficacy of a multiplexed gene panel approach for the detection of the molecular causes of neurodegenerative diseases with high genetic heterogeneity.

## Collaborative gene identification strategies in hereditary ataxias

Rebecca Schule[1, 2, 6], Matthis Synofzik[2, 6], Michael Gonzalez[1], Rafael Acosta[1], Katrien Smets[3, 4], Charles Lourenco[5], Wilson Marques[5], Peter de Jonghe[3, 4], Ludger Schöls[2, 6], Stephan Zuchner[1]

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**Background**: Recessive ataxias are genetically remarkably heterogeneous. Mutations in the genes encoding frataxin, polymerase gamma and sacsin collectively explain about 40% of cases. Mutations in the remaining about 70 recessive ataxia genes are extremely rare and each explain <1% of cases. Therefore currently ~50% of families with recessive ataxia remain without a genetic diagnosis.Although whole exome sequencing has dramatically facilitated the identification of novel genes, the genetic heterogeneity of ataxias, small pedigrees, limited cohort sizes due to the overall rarity of the disease and abundance of rare genomic variation are still limiting factors.

**Methods**: We have developed the GEnomes Management Application GEM.app (www.analysis-grid.net) to make large-scale genomic analysis and collaborative data sharing available to users of all technical backgrounds. The user friendly graphical user web interface combined with a highly distributed computing cluster allows real time analysis of the about 6000 whole exomes and ~900 whole genomes currently in GEM.app. Pre-defined configurable queries facilitate the analysis of variants within families as well as across cohorts.

**Results**: Within our collaborative network spanning five continents we have collected > 150 families with early onset recessive ataxias and performed whole exome sequencing on the index cases and in some cases additional family members. After exclusion of mutations in known ataxia genes, candidate genes carrying rare likely deleterious variants co-segregating with the disease in affected families were selected; independent evidence for involvement of these candidate genes in the pathogenesis of ataxia was then collected by screening of a second cohort of sporadic early onset ataxia patients. Comparison of variant frequencies in ataxia samples compared to non-ataxia phenotypes in GEM.app further allowed prioritization of candidate genes and variants. This approach has led to the identification of several novel recessive ataxia genes, including GBA2(1), PNPLA6(2), and KIF1C(3). Genetic and functional studies on further candidate genes are ongoing.

**Conclusions**: Collaboration and data sharing are essential to reduce time to discovery in rare disease research and allow resolving increasingly complex research questions. By allowing users of non-computational backgrounds to directly engage in exome and genome data analysis and to collaborate and safely share data on their families, GEM.app has facilitated the discovery of over 60 novel Mendelian disease genes within the past five years.

**References**:

1. Martin E…et al. Loss of function of glucocerebrosidase GBA2 is responsible for motor neuron defects in hereditary spastic paraplegia. Am J Hum Genet. 2013;92:238-44.
2. Synofzik M.…et al. PNPLA6 mutations cause Boucher-Neuhauser and Gordon Holmes syndromes as part of a broad neurodegenerative spectrum. Brain. 2014;137(Pt 1):69-77.
3. Caballero Oteyza…. et al. Motor protein mutations cause a new form of hereditary spastic paraplegia. Neurology. 2014.

## Exome and panel sequencing in a large cohort of early-onset ataxias: novel genes and genetic mechanisms

Matthis Synofzik[1, 2], Rebecca Schüle[1, 2, 3], Holger Prokisch[4], Robert Kopajtich[4], Tobias Haack[4], Tobias Bonifert[5], Bernd Wissinger[5], Stephan Züchner[3], Peter Bauer[6], Ludger Schöls[1, 2]

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**Background and aims:** Early onset ataxias (EOAs) are a highly heterogeneous group of degenerative and metabolic diseases, mostly caused by recessive mutations. Despite rapid progress in molecular genetics, the genetic basis and underlying biology of many EOAs still remains unsolved. Combining a multicentre international collaborative effort, we here aggregated a large cohort of EOA patients and a combination of different genetic methodologies to unravel novel genes and genetic mechanisms underlying the pathogenesis of EOA.

**Methods:** We performed a series of studies using a combined clinical, genetic and biological strategy: (i) a next-generation sequencing panel to identify all known and the most recent candidate EOA genes; (ii) comprehensive whole-exome sequencing of a large EOA cohort; (iii) automated high-throughput analysis pipeline already including n>200 EOA exomes; and, wherever possible, (iv) functional biological validation of novel EOA gene variants and mechanisms.

**Results:** Our blended strategy allowed us to identify and confirm four novel genes and genetic mechanisms associated with EOA. 1. Loss-of-function mutations in *DNAJC3*, a protein involved in the stress response of the endoplasmatic reticulum, cause EOA with widespread multisystemic neurodegeneration and diabetes mellitus, thus indicating a common pathway underlying both diabetes and neurodegeneration. 2. Mutations in *PNPLA6*, a protein de-esterifying the membrane protein phosphatidylcholine, cause EOA with hypogonadotropic hypogonadism and/or chorioretinal dystrophy (Boucher-Neuhäuser syndrome). *PNPLA6* causes this phenotypic cluster not as an isolated syndrome, but as part of broad continuous spectrum of neurodegenerative disease. 3. *STUB1*, where variants had been identified only in one Chinese EOA family so far, could be confirmed as a novel EOA gene. We identified 3 Caucasian ataxia patients with 4 novel missense mutations in *STUB1*, including 3 mutations in its tetratricopeptide-repeat domain. 4. *OPA1*, a well-established gene known to cause autosomal-dominant optic atrophy, could now be shown to cause complex EOA syndromes ("optic atrophy plus syndrome"; "Behr syndrome"), mimicking multisystemic autosomal-recessive ataxia. These complex syndromes result from the combined mutational effect of two *OPA1* variants occurring in *trans*.

**Conclusions:** Our results demonstrate the power of a large international collaborative effort to identify several novel EOA genes and genetic mechanisms within a short time-frame (<3 years).

## The identification of novel spinocerebellar ataxia disease genes using next generation sequencing approaches

Esther Nibbeling[1], Anna Duarri[1], Cleo Smeets[1], Corien Verschuuren-Bemelmans[1], Michiel Fokkens[1], Bart van de Warrenburg[2], Berry Kremer[3], Cleo van Diemen[1], Dennis Dooijes[4], Martijn Dijkstra[5], Juha Karjalainen[1], Morris Swertz[5], Lude Franke[1], Richard Sinke[1], Dineke Verbeek[1]

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To date, 37 different dominantly inherited spinocerebellar ataxia (SCA) types are known. Currently, genetic testing of the most frequent SCA genes via routine DNA diagnostic screening leaves 30% of the cases genetically undiagnosed. To further elucidate the genetic basis of this disorder, we used whole exome sequencing (WES) to identify the genetic cause of 20 small Dutch ataxia families, without mutations in the most common SCA genes.

WES was performed on 40 individuals preferably from two affected cousins per multiplex family if possible, or parent-child trios, with one affected parent and one affected child in simplex families. The data was prioritized using Ingenuity software focusing on the damaging variants including truncating-, missense- and splice site variants. All variants with a minor allele frequency higher than 0,1% were excluded. The remaining variants were prioritized for cerebellum and/or Purkinje cell expression. Finally, all selected variants were validated by Sanger sequencing and co-segregation analysis.

We could identify a single genetic cause in 7 families, including 5 families that carried mutations in the SCA6 (CACNA1A), SCA14 (PRKCG), SCA21 (TMEM240), and SCA40 (CCDC88C) genes. However, we identified multiple plausible candidates (n=39) in the remaining 13 families. In attempt to identify a candidate gene that was mutated in multiple families, we performed targeted resequencing of all genes in which variants segregated with the disease in these families, in 96 seemingly unrelated cerebellar ataxia singletons. These singletons also did not carry mutations in the most common SCA genes. The array also contained 14 genes known to be implicated in SCA that are currently not routinely screened in diagnostic setting.

Using this strategy, we identified in 15% of the cases in this singleton cohort mutations in the rarer known SCA genes including SCA5, 6, 11, 14, 15/16, 28, and 35. In 8 WES candidate genes, we identified one or multiple additional cases that carried likely pathogenic variants, and this confirmed their involvement in disease. These novel candidate genes account for 40% and 13.5% of the cases in the familial and singleton cohorts, respectively. These genes play a role in pathways known to be involved in cerebellar neurodegeneration such as neurogenesis and synaptic transmission. Additionally, cell-cell adhesion and chromatin remodeling seems to be important in the etiology of SCA.
Currently, we are functionally validating the impact of these mutations in in vitro models, to gain novel insights in the disease mechanisms underlying cerebellar neurodegeneration and ataxia. In conclusion, combining WES with targeted sequencing is an excellent method to identify new disease genes in small families.

## Surprising results from next generation sequencing: summary of >50 ataxia exomes sequenced

Margit Burmeister[1, 2, 3, 4, 5], Erin Sandford[1], Qianyi Ma[2], A. Bilge Ozel[2], Bo Li[2, 3], Randi Burns[1, 4], Jacqueline Fontaine[1], Jun Li[1, 2, 3]

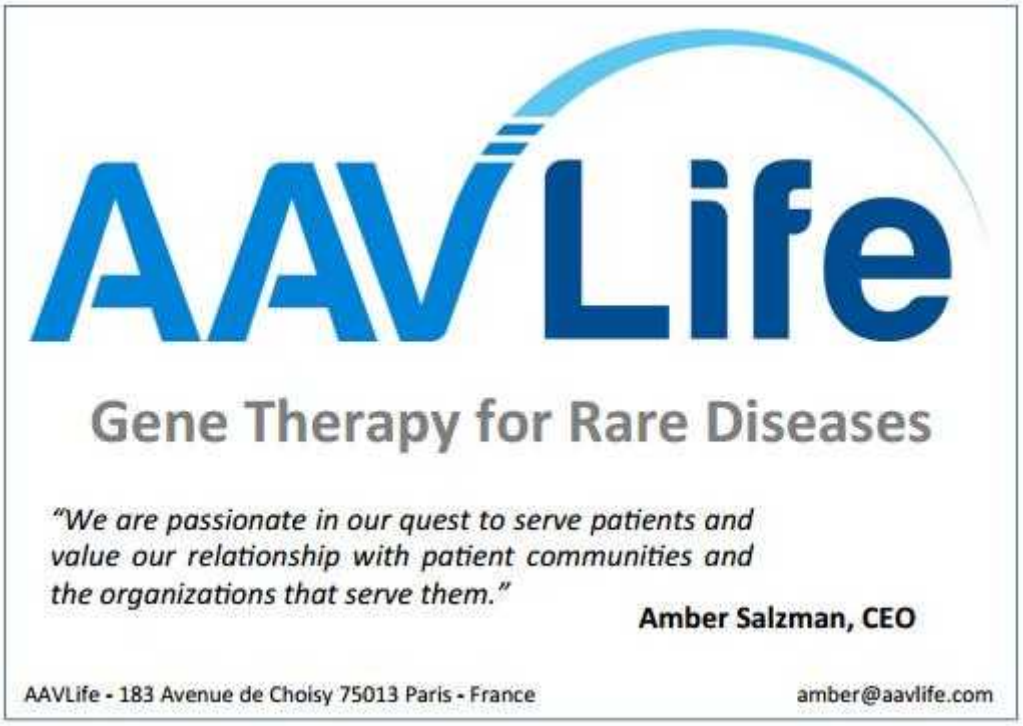
*[1] Molecular & Behavioral Neuroscience Institute, University of Michigan, United States, [2] Department of Human Genetics, University of Michigan, United States, [3] Department of Computational Medicine & Bioinformatics, University of Michigan, United States, [4] Cellular & Molecular Biology Graduate Program, University of Michigan, United States, [5] Department of Psychiatry, University of Michigan, United States*

Next Generation Sequencing has started to revolutionize diagnosis in medicine. In order to identify novel ataxia genes, for the past 20 years, our laboratory has recruited families with undiagnosed ataxias who have tested negative on the appropriate clinical genetic tests. With the help of animal models and additional families, we have identified several new ataxia genes: *KCND3* in dominant (Ann Neurology 2012), and *CWF19L1* in recessive ataxia (Neurology 2014). Several novel candidate genes involved in autophagy, transcription, ubiquinone, and vesicular sorting, are still being evaluated functionally *in-vitro* or in animal models and will be discussed. Consistent with several recent publications, we also found *ANO10* mutations in adolescent/adult onset recessive ataxia, confirming this is a fairly common form of ataxia. Interpretation of “variants of unknown significance” was inconsistent between medical providers, and may lead to unnecessary and expensive testing in search of alternative diagnosis. We identified mutations in *POLR3B* in a sibship with ataxia. *POLR3B* mutations cause hypomyelinating leukodystrophy with oligodontia and hypogonadic hypogonadism, also known as 4H syndrome. Upon re-contact, these ataxia patients had a phenotype consistent with 4H syndrome that was overlooked during original referral.Recognizing repeat expansions in exome sequencing data is particularly difficult. We have developed an algorithm to use exome sequencing data of >101 bp paired end reads to identify potential repeat expansions, and found an *ATXN8OS* repeat expansion in a sibship in which clinical tests had excluded *SCA8*. PCR across the gene confirmed the algorithm-predicted repeat expansions.

Summary: Next generation sequencing has revolutionized diagnostics in ataxia, and much more will be learned in the coming years when new genes and pathways will be identified.

- Validity of clinical genetic testing may be undermined by both testing errors and uncertain interpretation.
- Subjects and their physicians may not recognize the presence of other symptoms pointing to syndromic forms of ataxia that remain unrecognized, but may be clinically important or even actionable.
- Some progress has been made in identifying repeat expansions in exome data.
- The path from a list of genetic variants that might be involved in ataxia to confirmation of such results is difficult. Our laboratory is very interested in sharing results with others to help solidify novel candidate genes.

We thank the subjects and their families, referring clinicians for participation, and the NIH for funding (R01 NS078560).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# Session 2: Genetic and molecular mechanisms of ataxias

## Thursday 26th March

*Chairs: Sanjay Bidichandani (University of Oklahoma, US) and Olaf Riess (University of Tubingen, Germany)*

Invited speakers

8:30 Sanjay Bidichandani (University of Oklahoma, USA):
*Epigenetic Promoter Silencing in Friedreich ataxia*

9:00 Steve Jackson (University of Cambridge, UK):
*Assembly and disassembly of protein complexes at sites of DNA damage: mechanistic insights and therapeutic applications*

Selected presentations

9:30 Eleonora Di Gregorio (University Hospital, Torino, Italy):
*Unraveling molecular pathogenesis of SCA38, a novel autosomal dominant ataxia*

9:45 Conceição Bettencourt (UCL Institute of Neurology, UK):
*Transcriptome-wide analysis of the human brain as a route to SCA pathways and biomarkers*

10:00 Marguerite Evans-Galea (Murdoch Childrens Research Institute, Melbourne, Australia):
*The impact of compound heterozygous mutations in FXN on clinical outcome in Friedreich ataxia: insights from frataxin structure and function*

10:15 Natalia Gromak (Sir William Dunn School of Pathology, University of Oxford, UK):
*R-loop function in pathology of Friedreich ataxia and implications for other expansion disorders*

10:30 Break

11:00 Arnulf Koeppen (VA Medical Center Albany, NY, USA):
*Cardiac remodeling in Friedreich ataxia*

11:15 Marek Napierala (University of Alabama at Birmingham, US):
*Expanded GAA repeats induce transcriptional silencing restricted to the FXN locus and decrease the elongation rate through the FXN gene*

11:30 Thorsten Schmidt (University of Tuebingen, Netherlands):
*Targeting the intracellular localization of ataxin-3 as a road to therapy of Spinocerebellar Ataxia Type 3 (SCA3)*

11:45 Ana M Silva (University of Oxford, UK):
*Expanded GAA repeats impair frataxin gene expression and promote repositioning to the nuclear periphery at single-cell level*

12:00 Ana Teresa Simões (University of Coimbra, Portugal):
*Identification of the calpain cleavage sites in ataxin-3 protein*

12:15 Michael Themis (Brunel University, UK):
*Lentivirus mediated FXN gene delivery restores genome stability and DNA damage repair potential in human and mouse FRDA fibroblasts*

12:30-12:45 Close of session

Invited Speaker: Sanjay Bidichandani (University of Oklahoma, US)

## Epigenetic promoter silencing in Friedreich ataxia

Bidichandani S.I., Chutake Y.C., Lam C., Costello W.N.

[1] *Department of Pediatrics, University of Oklahoma Health Sciences Center, USA*

The expanded GAA triplet-repeat mutation in Friedreich ataxia causes transcriptional deficiency via epigenetic silencing of the FXN promoter. The ensuing defect of transcriptional initiation, which correlates with the length of the GAA triplet-repeat mutation, is the major cause of transcriptional deficiency in Friedreich ataxia. Epigenetic promoter silencing in Friedreich ataxia is mediated by altered nucleosomal positioning, which obliterates the normal nucleosomal depleted region at the FXN transcriptional start site. A class I HDAC inhibitor, 109/RG2833, currently being developed as a rational therapy for Friedreich ataxia, increased FXN promoter accessibility upon assaying individual chromatin fibers via NOMe-Seq analysis. Metabolic labeling of nascent transcripts revealed that 109/RG2833 significantly improved FXN promoter function in patient-derived cells. Epigenetic promoter silencing in Friedreich ataxia is therefore reversible, and these data implicate class I HDACs in repeat-mediated epigenetic promoter silencing.

*This research was made possible by a grant from the NIH to SIB (R01 NS072418)*

Invited Speaker: Steve Jackson (University of Cambridge & the Sanger Institute, UK)

## Assembly and disassembly of protein complexes at sites of DNA damage: mechanistic insights and therapeutic applications

At rest, each human cell sustains tens of thousands of DNA lesions per day. Furthermore, additional DNA damage can be induced by a range of agents, perhaps the most notable of which is ionizing radiation. To combat such threats to genome stability, our cells have evolved elaborate ways to detect, signal the presence of and repair DNA damage. The importance of such processes is highlighted by inherited or acquired defects in them causing various human pathologies, including immune-deficiencies, neurodegenerative diseases and cancer. Moreover, our increasing knowledge of cellular DNA-damage responses is providing exciting opportunities for developing novel classes of drugs to treat cancer and other age-related diseases. Work in my laboratory aims to decipher the mechanisms by which cells respond to the most toxic of all DNA lesions – DNA double-strand breaks (DSBs) – with much of our work addressing the functions of proteins that mediate such mechanisms, and the generation and molecular functions of post-translational modifications (PTMs) on DSB-responsive proteins. In this talk, I will highlight some of our recent work showing how a combination of PTMs control important molecular transitions in DDR-protein complex assembly and disassembly, and how such events promote genome stability. I will explain how this work is providing new approaches for treating cancer. Furthermore, I will discuss how our ongoing work might also yield opportunities for treating ataxias.

*References*


1. Jackson, S.P. and Bartek, J. (2009). The DNA-damage response in human biology and disease. ***Nature*** 461, 1071-1078.
2. Jackson, SP. (2009). The DNA-damage response: new molecular insights and new approaches to cancer therapy. ***Biochem. Soc. Trans***. 37, 383-394
3. Polo SE and Jackson SP. (2011). Dynamics of DNA damage response proteins at DNA breaks: a focus on protein modifications. ***Genes Dev.*** 25, 409-433.

## Unraveling molecular pathogenesis of SCA38, a novel autosomal dominant ataxia

Eleonora Di Gregorio[1], Marta Ferrero Ferrero[2], Neftj Ragusa[2], Elisa Giorgio[2], Cecilia Mancini[2], Simona Cavalieri[1], Elisa Pozzi[2], Chiara Costanzi[3], Loredana Boccone[4], Nico Mitro[5], Donatella Caruso[5], Barbara Borroni[3], Alfredo Brusco[1, 2]

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Spinocerebellar ataxia type 38 (SCA38) is a rare form of ataxia characterized by a pure cerebellar phenotype with slow progression. Cerebellar atrophy without obvious brainstem involvement in the moderate-severe stages and a clear-cut cerebellar hypometabolism at FDG-Positron Emission Tomography (FDG-PET) in the mid disease stages are present.

We mapped SCA38 locus at chromosome 6p and causative mutations were identified in ELOVL fatty acid elongase 5 gene (ELOVL5). To date two missense mutations are known: c.689G>T (p.Gly230Val) in three Italian families and c.214C>G (p.Leu72Val) in a French family.

ELOVL5 belongs to a multigenic family of genes encoding elongases and is involved in the synthesis of Poly Unsaturated Fatty Acids (PUFA) of both the ω3 and ω6 series (arachidonic acid (20:4, ω6), eicosapentaenoic acid (EPA, 20:5, ω3) and docosahexaenoic acid (DHA, 22:6, ω3)).

We demonstrated that the identified mutations caused subcellular mislocalization of ELOVL5 in four different cell types (COS-7; SK-N-BE2, NIH/3T3 and HeLa cells). The wild type protein localized in the endoplasmic reticulum (ER) compartment as expected by published data. In contrast, p.Leu72Val and p.Gly230Val ELOVL5 showed a less diffuse ER signal, with a perinuclear and polarized subcellular localization, consistent with an increase in the Golgi apparatus.

We focused our study on the impact of missense ELOVL5 changes on protein misfolding and the activation of unfolded protein response (UPR). Our preliminary data demonstrated a significant increased of the transcription factor CHOP, an UPR marker, in p.Gly230Val transfected cells. The p.Leu72Val aminoacidic substitution only slightly enhanced CHOP. Overall, our data showed the p.Gly230Val substitution was causing a mislocalization of the protein and might activate UPR. We will plan to treat cells expressing mutant ELOVL5 with MG-132, a membrane-permeable proteasome inhibitor, or 10% glycerol, a chemical chaperone known to stabilize the unfolded proteins ELOVL5 in order to study the impact of missense mutations on the protein conformation. If missense mutations cause a misfolded ELOVL5 protein, we will expect an increased protein level (detected by Wb) in MG-132 and 10% glycerol treated cells carrying the mutations. These experiments will clarify if missense mutations in ELOVL5 are engaging a toxic gain of function mechanism by an accumulation of unfolded protein in SCA38. Moreover we are investigating the UPR activation in Elovl5-/- mice embryonic fibroblasts transfected with aberrant ELOVL5 expressing vectors.

Because the main product of ELOVL5 is DHA and its level regulates ELOVL5 expression by a feedback regulatory mechanism, we have initial results showing that in vitro DHA treatment reduced ELOVL5 expression in lymphoblasts from three SCA38 affected subjects carrying the c.689G>T missense mutation.

Our preliminary data suggest that DHA might be used in patient treatment in order to reduce aberrant ELOVL5 and compensate the decreased omega-3 fatty acids in SCA38 patients.

## Transcriptome-wide analysis of the human brain as a route to SCA pathways and biomarkers

Conceição Bettencourt[1], Mina Ryten[1, 2], Paola Forabosco[3], Sebastian Guelfi[1, 2], Stephanie Schorge[4], Joshua Hersheson[1], UK Brain Expression Consortium (UKBEC)[1], John Hardy[1], Henry Houlden[1]

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Autosomal dominant spinocerebellar ataxias (SCAs) are clinically and genetically heterogeneous neurodegenerative diseases, all sharing progressive cerebellar dysfunction. More than 20 causative genes have been identified, but mutations in these genes explain only 50% to 60% of SCA cases. To date, no effective treatments exist, and the knowledge of drug-treatable molecular pathways is limited. The examination of overlapping mechanisms and the interpretation of how ataxia genes interact in the brain, as well as genes and pathways differentially expressed in the brain of patients, will be key for the discovery of additional genes, biomarkers, and potential disease-modifying agents. The main goal of this study was to address the possible relationships between known SCA genes in the human brain, predict their functions, and identify overlapping pathways that may be dysregulated in diseased brains, using whole-transcriptome expression analysis.

As part of the UK Brain Expression Consortium (UKBEC), we analysed the expression profile (Affymetrix Human Exon 1.0 ST arrays) of 788 brain samples obtained from 101 neuropathologically healthy individuals (10 distinct brain regions each). Weighted gene coexpression network analysis (WGCNA) was used to cluster 24 SCA genes into gene coexpression modules in an unsupervised manner. The overrepresentation of SCA transcripts in modules identified in the cerebellum was assessed. Functional enrichment analysis was performed to infer the functions and molecular pathways of genes in biologically relevant cerebellar modules. We also performed a pilot RNAseq study in post-mortem brain tissue from three SCA3 patients. The cerebellum, putamen, and frontal and occipital cortices were studied for these patients, and UKBEC RNAseq data from control individuals was available for the same brain regions. RNAseq data analysis, to determine differentially expressed genes between SCA3 patients and controls, and between brain regions, is ongoing.

WGCNA analysis revealed two cerebellar gene coexpression modules significantly enriched for SCA transcripts ($p = 0.021$, and $p = 2.87 \times 10^{-5}$) as well as for established granule and Purkinje cell markers (designated GC and PC, respectively). The GC module includes genes involved in the ubiquitin-proteasome system and contains SCA genes usually associated with a complex phenotype, while the PC module encloses many genes important for calcium homeostasis and signalling and contains SCA genes associated mostly with pure ataxia.

Using normal gene expression in the human brain, we identified cell types and pathways relevant to SCA pathogenesis. The overrepresentation of genes involved in calcium homeostasis and signalling suggests this pathway as an important target for therapy. These networks also provide valuable lists of candidate genes for diseases with overlapping phenotypes, and good candidates for novel genetic modifiers. The RNAseq analysis on SCA3 patients will provide further insights on dysregulated pathways in diseased brains, and new avenues for the search of biomarkers and therapeutic targets.

## The impact of compound heterozygous mutations in *FXN* on clinical outcome in Friedreich ataxia: *insights from frataxin structure and function*

Charles Galea[1, 2], Aamira Huq[2], Paul Lockhart[2, 3], Geneieve Tai[2, 3], Louise Corben[2, 4], Eppie Yiu[2, 3, 5], Roger Peverill[6], Lyle Gurrin[7], David Lynch[8], Sarah Gelbard[8], Alexandra Durr[9], Michael Parkinson[10], Paola Giunti[10], Susan Perlman[11], Martin Delatycki[2, 3, 4, 12] , Marguerite Evans-Galea[2, 3]

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Friedreich ataxia (FRDA) is an autosomal recessive neurodegenerative disease characterised by incoordination and hypertrophic cardiomyopathy. The majority of individuals with FRDA have homozygous GAA trinucleotide repeat expansions in the first intron of FXN with reduced expression of the mitochondrial protein frataxin. The remaining affected individuals are compound heterozygous for a GAA expansion and a point or insertion and/or deletion mutation within FXN. This study examines disease-causing mutations and their impact on frataxin structure and function, and clinical outcome in FRDA. This includes the potential to bind iron and interact with iron sulfur cluster assembly proteins like IscU and IscS. Clinical information from 111 compound heterozygous individuals with FRDA (81 published and 30 previously unpublished cases) was collated and compared to clinical data from 131 individuals homozygous for GAA expansions. Molecular modelling, in silico protein stability analyses using different prediction algorithms, and systematic review of published experimental data, were used to estimate the impact of each mutation on frataxin structure, function and stability. Each mutation was categorised into one of four groups: three compound heterozygous mutation groups (i) null – no frataxin produced, (ii) moderate/strong impact on protein stability/function and, (iii) minimal impact on protein stability/function; and (iv) homozygous GAA expansions. The difference in age of disease onset between the four categories was examined using regression analysis taking into account GAA expansion size(s). The likelihood of developing cardiomyopathy and diabetes between the mutation categories, taking the expansion into account, was also examined. Individuals with null mutations had a significantly earlier age of onset when compared to homozygous GAA expansions, after accounting for expansion size.

Comparison of the GAA expansion homozygous group to the minimal impact and moderately or strongly destabilising groups, found no significant differences in age of onset. Interestingly, individuals with homozygous GAA expansions had a greater likelihood of developing cardiomyopathy than individuals in all three compound heterozygous mutation categories. In contrast, those with null mutations were more likely to have diabetes mellitus than those with homozygous GAA expansions. Importantly, in compound heterozygous individuals we find that, in addition to frataxin expression from an expanded allele, expression of a mutant frataxin with partial function is of greater benefit than no frataxin expression. This study provides a foundation for further analyses of the different mutations in frataxin to better understand the link between structure and function, and clinical outcome in FRDA.

## R-loop function in pathology of Friedreich ataxia and implications for other expansion disorders

Natalia Gromak, Matthias Groh, Lara Silva

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Around forty human diseases are associated with expansion of small nucleotide sequences. Depending on their location, these expansions can either lead to expression of toxic proteins or transcriptional repression/gene silencing of the host gene. In Friedreich ataxia (FRDA), caused by an expanded (GAA)n repeat sequence in intron 1 of the frataxin (FXN) gene, transcriptional repression has been proposed to be associated with the formation of unusual DNA sequences and repressive chromatin over expanded gene. In our lab we established a DNA immuno-precipitation method (DIP), which allowed us to detect RNA/DNA hybrids (R-loops) in human cells (Skourti-Stathaki et al, 2011). Recently, using DIP we showed that R-loops are formed on expanded FXN alleles and trigger the formation of repressive chromatin in cells from FRDA patients (Groh et al, 2014). We further investigated the molecular mechanism of R-loop-mediated heterochromatin formation in FRDA. In particular, we studied the contribution of RNAi machinery to this process. Our results demonstrate that methyl-transferase G9a, which deposits H9K9me2 mark, is specifically enriched over expanded GAA repeats. Interestingly, we observed the recruitment of RNAi machinery at the promoter region of the FXN gene, which was reduced in FRDA cells. This suggests that R-loops are likely to trigger heterochromatin formation in RNAi-independent manner. We will present the molecular details of this process and its contribution to the pathology of other expansion disorders.

*References:*

K Skourti-Stathaki, N. J. Proudfoot and N. Gromak. 'Human senataxin resolves RNA/DNA hybrids formed at transcriptional pause sites to promote Xrn2-dependent termination'. Molecular Cell (2011) 42(6), 794-805.

M.Groh, M.Lufino, R.Wade-Martins, N.Gromak. R-loops formed over expanded repeats cause transcriptional silencing in Friedreich ataxia and Fragile X syndrome. PLOS Genetics 10 (5) (2014).

M.Groh and N.Gromak. Out of balance: R-loops in human disease. PLOS Genetics (2014)

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## Cardiac remodeling in Friedreich ataxia

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Heart failure is the most frequent cause of death in patients with Friedreich ataxia (FA). Pathological examination reveals many abnormalities, including concentric hypertrophy (occasionally also dilated cardiomyopathy); accumulation of iron (Fe) in cardiomyocytes, accelerated biosynthesis of ferritin and mitochondrial ferritin, inflammatory infiltration of the endomysium, invasion of cardiomyocytes by monocytes, fiber necrosis, and phagocytosis of myofibrils. Abnormalities of intercalated discs (ID) in the main working myocardium of left ventricular wall (LVW), right ventricular wall (RVW), and ventricular septum (VS) contribute to mechanical cardiac dysfunction and arrhythmogenicity in FA. In normal hearts, desmosomes and fascia adherens junctions of ID provide the mechanical connection between tubular heart fibers. ID are also the main sites of gap junctions that serve electrochemical conductivity between heart fibers. We examined 14 autopsy specimens and one cardiac biopsy of FA and 12 normal controls by immunohistochemical methods and laser scanning confocal immunofluorescence microscopy to assess the structural changes of ID. Antibodies to N-cadherin, a robust marker of the fascia adherens complex, visualized an overall paucity of ID in all three anatomical sites of the heart in FA, abnormally large ID sizes, scalloping, duplication, and fragmentation. Similar results were obtained with an antibody to vinculin, an ID protein with the putative function of linking actin filaments to fascia adherens junctions. Immunohistochemistry of desmoplakin, an essential component of the desmosomal complex, yielded comparable reaction product. Semi-thin plastic-embedded sections of LVW revealed excessive undulations of the abutting sarcoplasmic membranes of ID, and on longitudinal sections, the distances between ID of FA hearts were significantly increased (in µm; mean ± standard deviation; N=15): FA, LVW: 75.1±7.4; controls (N=12), LVW: 57.5±11.4; FA, RVW: 77.8±14.7; controls, RVW: 54.6±10.1; FA, VS: 79.6±11.4; controls, VS: 49.3±7.6. The distribution of connexin 43 and ZO-1, markers of gap junctions in the main working myocardium, was also abnormal. Immunofluorescence of connexin 43 matched the disorganized ID in FA, but the gap junction protein also showed lateralization to areas beyond the ID, such as cardiomyocyte plasma membranes and the sarcoplasm. In conclusion, the pathogenesis of FA cardiomyopathy includes an inflammatory necrotizing component and remodeling of ID and gap junctions. The result is a combination of reduced contractile tissue, inefficient mechanical coupling, and incorrect distribution of ion channels. It remains to be determined how frataxin deficiency causes the varied pathological phenotype of the heart in FA (Supported by Friedreich's Ataxia Research Alliance, National Institutes of Health, and Neurochemical Research, Inc.).

## Expanded GAA repeats induce transcriptional silencing restricted to the FXN locus and decrease the elongation rate through the FXN gene

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Friedreich's ataxia (FRDA) is a severe neurodegenerative disease caused by transcriptional repression, which is induced by expanded GAA repeats located in intron 1 of the FXN gene. FRDA patients homozygous for GAA expansion have 5 – 30 % of frataxin mRNA and protein when compared with healthy individuals. It has been demonstrated, using various model systems along with patients' autopsy samples, that expanded GAA repeats induce epigenetic silencing of the FXN gene. Postranslational histone modifications that typify heterochromatin are enriched in the vicinity of the repeats, while active chromatin marks in this region are underrepresented in FRDA samples when compared to controls. Thus far, silencing triggers as well as the exact molecular mechanism of FXN silencing remain unknown.

Spreading of heterochromatin and transcriptional silencing has been observed in the proximity of the highly repetitive regions of the genome such us centromeres. However, the extent of silencing induced by expanded GAAs beyond the direct vicinity of intron 1 has not been determined. Results indicating both silencing of the FXN promoter associated with a transcription initiation defect as well as studies demonstrating transcription elongation dysfunction have been reported. Moreover, recent phenotypic observations conducted mostly in FRDA patient-derived fibroblast and lymphoblast cell lines indicate the possibility of a long-range cis silencing mechanism, spanning a larger region of the FXN locus. The extent of GAA repeat-induced silencing is of particular importance considering two important therapeutic strategies for FRDA: reversal of FXN silencing and gene replacement therapy. In the case of extensive transcriptional silencing of a larger region of chromosome 9, restoring frataxin expression using gene therapy strategies would alleviate only the part of the disease phenotype arising solely from frataxin deficiency. On the other hand, strategies based on epigenetic reactivation of the FXN locus should result in complete reversal of FRDA phenotype. However, if the effect of GAA expansion is localized to the FXN gene, both therapeutic avenues should be equally efficacious.

We present the results of a comprehensive analysis of the transcription status and epigenetic environment of the FXN locus in a large set of 17 primary fibroblast cell lines derived from FRDA patients and 18 lines obtained from unaffected controls. Using next-generation RNA sequencing, we demonstrated a remarkable variability in FXN expression within both FRDA as well as control samples. Additionally, we showed that the epigenetic silencing effect induced by the expanded GAA repeats is confined to the FXN locus and does not affect expression of upstream or downstream neighboring genes. Finally, analysis of FXN pre-mRNA expression between FRDA and control samples revealed a pronounced transcription elongation defect at the expanded GAA region.

## Targeting the intracellular localization of ataxin-3 as a road to therapy of Spinocerebellar Ataxia Type 3 (SCA3)

Thorsten Schmidt[1, 2], Zijian Wang[1, 2], Anna Sowa[1, 2], Maria Inês Martins[1, 2], Andreia Teixeira de Castro[3], Patrícia Maciel[3], Noomen Bettaga[1, 2], Paul Antony[1, 2], Olaf Riess[1, 2]

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Spinocerebellar ataxia type 3 (SCA3) or Machado-Joseph disease (MJD) is a neurodegenerative disorder caused by a CAG expansion in the *MJD1* gene leading to a polyglutamine expansion in the encoded Ataxin-3 protein. In controls, Ataxin-3 is predominantly located in the cytoplasm but forms protein aggregates in the nucleus of neurons in SCA3 patients. We recently demonstrated *in vivo* that the toxicity of expanded Ataxin-3 is linked to its intracellular localization: Targeting Ataxin-3 to the nucleus gave rise to a strong phenotype with a high number of protein aggregates. Purely cytoplasmic Ataxin-3, however, even with a highly expanded polyglutamine repeat (148 glutamines), was not able to induce a phenotype and even did not aggregate. In addition, we identified and characterized intracellular transport signals (two nuclear export signals, NES, and one nuclear localization signal, NLS) within the coding sequence of Ataxin-3. Therefore, it is evident that proteins involved in the nucleocytoplasmic transport machinery recognize these localization signals, control the intracellular localization of Ataxin-3, thereby influence the toxicity and aggregation of Ataxin-3 and, thus, the pathogenesis of SCA3/MJD.

As pathologically Ataxin-3 remains harmless as long as it is kept in the cytoplasm, we anticipated the intracellular localization of Ataxin-3 as a target for a possible therapeutical intervention. For this reason, we generated an assay allowing us to easily monitor the intracellular localization of normal or expanded Ataxin-3. As it was demonstrated before that heat shock leads to a nuclear translocation of Ataxin-3 we used heat shock treatment to validate the efficacy of our assay. We then used our assay to screen a library of FDA-approved compounds, indeed identified compounds impacting the nuclear translocation of Ataxin-3 and validated them *in vivo*. As the compounds we identified are already FDA-approved and on the market, they could be transferred to the clinics comparatively fast. We believe that our results will improve the understanding of pathological mechanisms influencing the progression of the disease and are an important contribution towards a treatment of SCA3/MJD.

## Expanded GAA Repeats Impair Frataxin Gene Expression and Promote Repositioning to the Nuclear Periphery at Single-Cell Level

Ana M Silva[1, 2], Jill M Brown[3], Veronica J Buckle[3], Richard Wade-Martins[1], Michele Lufino[1]

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In Friedreich's Ataxia (FRDA), abnormal GAA trinucleotide–repeat expansions in intron 1 of the frataxin gene (FXN) cause epigenetic changes and reduce FXN mRNA levels in averaged cell samples though a poorly understood mechanism. Dissecting the silencing mechanism in FRDA in situ, through the analysis of FXN nuclear localisation and expression at single-cell level, is crucial to improve our understanding of the disease.

Here, we have developed a human cell model to analyse the link between FXN nuclear localisation and expression in single cells. FXN-MS2-Luc and FXN-GAA-MS2-Luc stable human clones carry a site-specific integration of a single copy of the whole FXN locus with either 6 or ~310 GAA repeats in intron 1, respectively. To fluorescently label the transgenic FXN mRNA, we inserted MS2 protein–binding sites into exon 2 by homologous recombination. The ~310 GAA repeat expansion in the FXN-GAA-MS2-Luc cell line recapitulates the characteristic FXN gene repression in FRDA.

FXN transgene localisation in the FXN-MS2-Luc and FXN-GAA-MS2-Luc lines was determined by DNA FISH. FXN was positioned at the nuclear periphery (NP) in ~44% of the FXN-GAA-MS2-Luc cells compared to ~10% of FXN-MS2-Luc cells. Restoring histone acetylation repositioned FXN-GAA-MS2-Luc away from the NP. To further understand FXN repression, we analysed the transcriptional output of individual transgenic FXN alleles by RNA FISH. FXN-GAA-MS2-Luc cells contained ~5 ± 2 mRNAs per cell and FXN-MS2-Luc cells contained ~9 ± 4 mRNA per cell, therefore ~310 GAA repeats reduce the number of mature mRNA molecules by 44% at single-cell level.

localisation was next analysed in its native genomic environment in carrier, healthy and FRDA patient cells. In carrier cells, the expanded FXN allele localised preferentially closer to and contacted more frequently with the NP than the normal allele. In FRDA versus healthy cells, the GAA expansion increased the probability of an allele to be found at the NP and consequently its probability of being silenced. Moreover, when expanded GAA-FXN alleles did express, it was at significantly reduced levels both in the nucleoplasm and especially at the periphery. We provide evidence that the combined effect of expansion with peripheral relocation results in a catastrophic reduction in expanded FXN transcriptional output, demonstrating a clear link between expanded FXN positioning at the NP and GAA-mediated transcriptional repression.

Collectively, these results suggest repressive epigenetic modifications at the expanded GAA-FXN locus may lead to NP relocation, where further repression may occur.

## Identification of the calpain cleavage sites in ataxin-3 protein

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Machado-Joseph disease, also known as spinocerebellar ataxia type 3 (MJD/SCA3), is the most frequent autosomal dominantly-inherited ataxia worldwide. Over-repetition of a CAG trinucleotide in the MJD1 gene translates into a polyglutamine tract within the ataxin-3 protein, a protein implicated in cellular quality control. According to the toxic fragment hypothesis, neurotoxicity might derive from the proteolysis of the host protein to liberate a polyglutamine fragment, an event suggested to be the trigger of the aggregation process, a hallmark of the disease. Calpains are calcium-dependent cysteine proteases, which mediate the formation of toxic fragments, ataxin-3 translocation to the nucleus and neurodegeneration.

The aim of this work was to understand in vivo which are the calpain cleavage sites in ataxin-3 protein. For that, we used site-directed deletion mutagenesis. Five amino acids around 3 putative cleavage sites i) “A”, ii) “B”, and iii) “C” were deleted individually in wild-type and mutant forms of ataxin-3 to produce calpain-resistant constructs. As a positive control, truncated forms of ataxin-3 considering the aforementioned cleavage sites were also designed. These constructs were expressed by lentiviral vectors transduction of the adult mouse brain. Western-blot analysis suggests that the mutation at amino-acid “A” in wild-type ataxin-3 can abrogate the production of the ~26 kDa fragment by 53%, while mutation at amino-acids “B” and “C” in mutant ataxin-3 decreased the production of the ~34 kDa and ~26 kDa fragments, respectively, by 63% and 57%. Which generated calpain cleavage fragment is more toxic contributing to ataxin-3 nuclear localization, aggregation and neurotoxicity still remains to be investigated. The understanding of the calpains role in MJD can further contribute to the comprehension of other ataxias vulnerable to calcium deregulation.

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## Lentivirus mediated FXN gene delivery restores genome stability and DNA damage repair potential in human and mouse FRDA fibroblasts

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Friedreich ataxia (FRDA) is a progressive neurodegenerative disease with primary sites of pathology in the large sensory neurons of the dorsal root ganglia (DRG) and dentate nucleus of the cerebellum. FRDA is also often accompanied by severe cardiomyopathy and diabetes mellitus. FRDA is caused by loss of frataxin (FXN) expression, which is due to GAA repeat expansion in intron 1 of the FXN gene. Frataxin is a mitochondrial protein important to iron-sulphur (Fe-S) cluster biogenesis and the electron transport chain (ETC). As a consequence of impaired mitochondrial energy metabolism, FRDA cells show increased levels of and sensitivity to oxidative stress, which is known to be associated with genome instability. In this study, we investigated DNA damage/repair in relation to FXN expression via immunostaining of gH2AX a nuclear protein that is recruited to DNA double strand breaks (DSBs). We found FRDA patient and YG8sR FRDA mouse model fibroblasts to have inherently elevated DSBs (1.8 and 0.9 foci/nucleus) compared to normal fibroblasts (0.6 and 0.2 foci/nucleus, in each case $p<0.001$). By delivering the FXN gene to these cells using a lentivirus vector (LV) at a copy number of ~1/cell, FXN mRNA and protein levels reached 270- and 202-fold, respectively to that of normal fibroblasts, without observable cytotoxicity. This resulted in a reduction in DSB foci to 0.7 and 0.43 (in each case $p<0.001$) in human and YG8sR fibroblasts, respectively and an increase in cell survival to that found for normal fibroblasts. We next irradiated the FRDA fibroblasts (2Gy) and measured their DSB repair profiles. Both human and mouse FRDA fibroblasts were unable to repair damaged DNA. However, repair returned to normal levels following LV FXN gene transfer. Our data suggest frataxin may be important for genome stability and cell survival. We are currently investigating whether lack of DNA damage repair in FRDA to be a factor that influences neurodegeneration.


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# Session 3a: Cellular and molecular models of Friedreich's ataxia

## Thursday 26th March

*Chairs: Helene Puccio (Inserm, Illkirch, France) and Michele Lufino (University of Oxford, UK)*

Invited speakers

14:00 Helene Puccio (Inserm, Illkirch, France):
*Progress in the development of new mouse and cell models for deciphering the neurological defects in Friedreich ataxia and testing therapies*

14:25 Michele Lufino (University of Oxford, UK):
*Cell and animal FXN genomic reporter models of Friedreich's ataxia*

Selected presentations

14:45 Vijayendran Chandran (University of California, Los Angeles, US):
*Inducible and reversible frataxin knock-down mouse model for Friedreich's ataxia*

15:00 Simona Donatello (University Libre de Bruxelles, Belgium):
*Induced pluripotent stem cell-derived neurons from Friedreich's ataxia patients have a cellular phenotype that can be reversed by frataxin inducers*

15:15 Jordi Magrane (Brain and Mind Research Institute, New York, US):
*Analysis of mitochondrial dynamics in cultured sensory neurons and in in vivo mouse models of Friedreich's Ataxia*

15:30 Juan Antonio Navarro Langa (University of Regensburg, Germany):
*Impact of frataxin-deficiency on mitochondrial dynamics*

15:45 Jose V. Llorens (University of Uppsala, Sweden):
*A new Drosophila melanogaster model to identify genetic modifiers of transcriptional repression caused by GAA expansion in FX*

16:00 Close of session

16:15 Coaches leave for Oxford

17:15-19:15 Free time

19:15-19:45 Drinks reception at Oxford Town Hall

19:45 Dinner in Oxford Town Hall
There will be presentations by Jeffrey Sherman (Horizon Pharma) who are sponsoring this event and Kyle Bryant (FARA) who has Friedreich's ataxia and is the Founder and director of Ride ataxia.

Invited Speaker: Helen Puccio (Inserm, Illkirch, France)

**Progress in the development of new mouse and cell models for deciphering the neurological defects in Friedreich ataxia and testing therapies**

Invited Speaker: Michelle Lufino (University of Oxford, UK)

## Cell and animal FXN genomic reporter models of Friedreich's ataxia

Michele Lufino[1], Ana Ferreira da Silva[1], Cioroch M[1], Angela Russell[2], Richard Wade-Martins[1]

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Currently, there is no treatment available for Friedreich's ataxia and the silencing mechanism induced by GAA expansions still needs further elucidations. The generation of cell and animal models which closely recapitulate the characteristic molecular features of FRDA is of great importance as it can facilitate the identification of promising therapies and improve our understanding of the FRDA pathogenesis. We have previously reported the generation of a FXN-GAA-Luc reporter cell model which carries a ~310 GAA repeats expansion within the context of the whole FXN genomic DNA locus, thus providing physiologically-relevant FXN expression. Here, we present an update on this model and the generation and use of two further reporter models, namely FXN-GAA-MS2 cells and FXN-GAA-Luc mouse model.

We have recently described the use of FXN-GAA-Luc cells to identify a novel FXN-increasing molecule, named C5, which is able to increase FXN expression in FRDA patient primary cells. Since C5 is characterized by a relatively high EC50, we have carried out structure-activity relationship (SAR) studies in order to identify compounds with improved frataxin up-regulating properties. We synthesized a series of derivatives of C5 and we tested their effect on frataxin protein levels using the FXN-GAA-Luc cell model. We report the identification of a new small molecule with an ~18-fold reduction in EC50 compared to C5, reducing the active concentration to the low uM range and representing a major improvement over to its parent molecule.

FXN-GAA-Luc cells provide an optimal readout for frataxin protein levels, however they do not represent a suitable tool to investigate the transcriptional kinetics of the FXN gene. To achieve this and to assess the effect of the GAA expansion of FXN transcription in live cells and at single-cell resolution, we modified the FXN-GAA-Luc cell model in order to utilize the MS2-based RNA imaging system. By performing Fluorescence Recovery After Photobleaching (FRAP), we show that the presence of a ~310 GAA repeat expansion greatly slows FXN transcriptional kinetics. Moreover, we demonstrate for the first time at single-cell resolution that expanded GAA repeats reduce FXN transcriptional output by inhibiting preferentially FXN transcription initiation.

Finally, we have recently developed a novel FXN-GAA-Luc mouse model for in vivo live visualization of frataxin protein levels and we show that that bioluminescence generated from the FXN-Luciferase fusion protein can be easily detected in live anesthetized animals. Since the light intensity represents a readout of frataxin protein levels, this model is particularly suitable for in vivo testing of frataxin-increasing therapies, allowing detection of frataxin levels before and after compound administration, therefore providing information on the extent and duration of frataxin up-regulating strategies.

In conclusion we describe a series of genomic-reporter models suitable for different applications.

## Inducible and reversible frataxin knock-down mouse model for Friedreich's ataxia

Vijayendran Chandran, Kun Gao, Revital Versano, Vivek Swarup, Daniel Geschwind

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Friedreich's ataxia (FRDA) is an early-onset neurodegenerative disease that progressively impairs motor function, leading to ataxic gait, cardiac abnormalities, and multiple other co-morbidities, ultimately resulting in early mortality (median age of death, 35 years). It is the most commonly inherited ataxia, and is caused by severely reduced levels of frataxin (Fxn) that usually result from a guanine-adenine-adenine (GAA) trinucleotide repeat expansion within the first intron of the Fxn gene. Animal models are valuable tools for mechanistic analysis and therapeutic development in FRDA. Existing transgenic and heterozygous knockout (homozygous are embryonically lethal) animal models, are either mildly symptomatic or restricted in their ability to recapitulate and evaluate the spatial and temporal aspects of FRDA pathology, as they are engineered to be tissue-specific conditional knockouts. We have developed an inducible mouse model for FRDA that permits reversible frataxin knockdown and detailed studies of the temporal progression or recovery following restoration of frataxin expression. We targeted a single copy shRNA against the Fxn transgene (doxycycline-inducible) under the control of H1 promoter gene into the rosa26 genomic locus. This allowed us to circumvent the lethal effect of organism-wide knockout, while permitting significant frataxin reduction in all tissues. Fxn knockdown was achieved to control the onset and progression of the disease depending on the dose of doxycycline (Dox). We observed ataxia, degeneration of dorsal root ganglia, scoliosis, and iron deposition, parallel to what is observed in FRDA patients. Rescue experiments were carried out by withdrawal of Dox to reverse the acceleration of disease progression even after significant motor dysfunction was observed. By controlling the onset and progression of the disease, and attempt rescue via restoration of frataxin levels, we aim to refine our understanding of the pathogenesis of FRDA and its reversibility at different stages of disability, assess biomarker response to drugs, and to test effective therapeutic agents.

## Induced pluripotent stem cell-derived neurons from Friedreich's ataxia patients have a cellular phenotype that can be reversed by frataxin inducers

Amélie Hu[1], Elisabeth Mangiameli[2], Ilaria Pelizzoni[2], Ana Oliveira[3], Myriam Rai[1], Decio L. Eizirik[3], Miriam Cnop[3], Mariana Igoillo-Esteve[3], Simona Donatello[3], Fabio Grohovaz[2], Franca Codazzi[2], Massimo Pandolfo[1]

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**Background and aims**: We employed induced pluripotent stem cell (iPSC)-derived neurons obtained from FRDA patients and healthy subjects to unveil phenotypic alterations related to frataxin deficiency and investigate if they can be reversed by treatments that upregulate frataxin. In a parallel study, we found that FRDA-neurons show mitochondrial oxidative stress-mediated activation of the intrinsic pathway of apoptosis, which can be prevented by cAMP-inducing drugs, such as GLP-1 agonists used in the treatment of type 2 diabetes. Here, we characterise alterations related to defective iron-sulfur cluster biogenesis and dysregulation of the oxidative stress response and iron handling. Our preliminary findings suggest that these alterations can be reversed by molecules that upregulate frataxin expression.

**Materials and Methods**: iPSCs were from two FRDA patients and two controls. Neurals cells were differentiated as previously described (Hick et al., 2012). We confirmed the stability of the GAA expansions throughout the differentiation process by PCR. Cellular identity at all stages of differentiation was determined by morphological analysis and by expression analysis of specific markers by immunifluorescence or by RT-PCR. Patch-clamp was used to assess electrophysiological properties. Protein levels were estimated by western blot. Videomicroscopy using specific fluorescent probes was used for iron and oxidative stress analyses.

**Results**: FRDA and control iPSCs were equally capable of differentiating into a neuronal or astrocytic phenotype. iPSC-derived neurons had a cortical phenotype and generated sodium currents and action potentials. We confirmed a slight delay of maturation of FRDA-neurons. The expression of two iron-sulfur proteins, the NDUFS3 mitochondrial Complex I subunit and mitochondrial aconitase and of two lipoic acid (synthesized by Fe-S proteins) -containing proteins, pyruvate dehydrogenase and alpha-oxoglutarate dehydrogenase was decreased by 25-50% in FRDA- vs. control-neurons. Conversely, the expression of mitochondrial superoxide dismutase was robustly increased in FRDA-neurons. FRDA-neurons showed a ± 5-fold increase in labile iron pool and a ± 2-fold decrease in reduced glutathione. Oxidative stress-mediated cell death after administration of 100 µM $H_2O_2$ was 5-fold higher in FRDA cells.

Treatment with the HDAC inhibitor 109 increased iron-sulfur protein levels, downregulated SOD2 levels and almost fully protected FRDA-neurons from oxidative stress-mediated cell death. Preliminary results suggest a similar effect of exendin-4.

**Conclusions**: iPSC-derived neurospheres from FRDA patients differentiate properly into neurons and astrocytes, though their neuronal functional maturation appears to be slightly delayed. FRDA-neurons show lower levels of iron-sulfur proteins, higher LIP and lower GSH levels, and enhanced sensitivity to oxidants compared to control-neurons, indicating deficient iron-sulfur cluster biogenesis, altered iron metabolism, and oxidative stress. Treatment with drugs that upregulate frataxin appears to reverse these phenotypic changes. Our findings suggest that correction of frataxin deficiency may not only stop disease progression, but also lead to clinical improvement by rescuing still surviving, but dysfunctional neurons.

## Analysis of mitochondrial dynamics in cultured sensory neurons and in *in vivo* mouse models of Friedreich's ataxia

Irene Bolea[1], Alex Gella[1], Wen-Biao Gan[2], Jordi Magrane[1]

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Friedreich's ataxia (FA) is a primary mitochondrial disorder which causes degeneration of different neuronal types, with a preference for large sensory neurons, cerebellar neurons, and corticospinal tracts. Very few studies, none of which in mammalian systems, have addressed the involvement of mitochondrial dynamics, such as axonal transport, fusion and fission, in FA neurons. Since work from us and others suggest that alterations of mitochondrial dynamics compromise neuronal function, synaptic activity, and the architecture of the cell, we hypothesize that abnormal dynamics may participate to the specificity of neuronal cell loss in FA.

We performed live imaging fluorescent microscopy in isolated sensory neurons and in the whole, living, mouse to examine mitochondrial morphology, distribution along axons, transport, and fusion and fission events. We have identified relevant pathogenic phenotypes for FA, such as fragmentation of mitochondria in axons and cell bodies as early as 4 days *in vitro* and impaired mitochondria bioenergetics, in sensory neurons of two different FA mouse models: the KIKO and the Sarsero mouse. Moreover, we have successfully imaged mitochondrial transport in the sural and femoral nerves of living FA mouse models after crossing them with the mitoDendra transgenic mouse. These *in vivo* studies allowed us to investigate mitochondria morphology and dynamics in affected and non-affected nerve tissues in longitudinal studies. We are currently investigating the cellular consequences of these mitochondrial abnormalities in neuronal architecture and function.

Our data provides a novel readout of FA pathology, which will allow us to evaluate therapeutic approaches targeting mitochondrial function and dynamics, and also to assess the efficacy of therapies that increase frataxin levels by monitoring the downstream consequences on mitochondria, and their impact on neuronal viability.

## Impact of frataxin-deficiency on mitochondrial dynamics

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Frataxin is a highly conserved mitochondrial protein that plays a major role in the biosynthesis of iron-sulfur clusters. Effects of frataxin downregulation in human samples and other disease models of Friedreich Ataxia (FA) include diminished activity of several mitochondrial enzymes, impaired ATP production and depolarization of mitochondrial membrane, among others. Remarkably, *Drosophila* models of FA have been able to reproduce all these biochemical features. As expected, frataxin-deficient flies display reduced membrane potential, aconitase activity and ATP levels as well as hypersensitivity to oxidative stress.

Moreover, mitochondria are dynamic organelles that fuse and divide according to the energetic demands of the cell. Therefore, we aimed to analyze whether cells suffering from an energy deprivation due to loss of frataxin would modify their mitochondrial network to compensate this defect. Indeed, alterations in mitochondrial morphology have been reported in FA models but this aspect has not been analysed in detail. Interestingly, we could show that frataxin deficiency affects mitochondrial morphology in glia. Our histological and molecular results also show a strong mitochondrial accumulation in an age and stress-dependent manner. Importantly, using the autophagy marker p62, we can conclude that mitochondrial accumulation in glia is mainly caused by an impaired degradation of damaged mitochondria. In agreement with this hypothesis, we also found that frataxin-deficient flies accumulate mtDNA. We are trying now to decipher the underlying mechanisms. Our results indicate that changes in the expression of *Drosophila mitofusin* (*dMfn*, a gene involved in mitochondrial fusion and degradation) might be a central event. In this sense, a genetic screen, carried out to find putative suppressors or enhancers of FA defects in *Drosophila*, revealed that *dMfn* downregulation is sufficient to counteract some of the frataxin-deficient phenotypes. Moreover, we have found that frataxin overexpression completely alters the mitochondrial network by triggering a strong clustering of mitochondria probably due to its capacity to increase ATP production. Our results link frataxin with the dynamic control of stability, integrity and homeostasis of mitochondria, providing new ideas for the development of potential therapeutic targets.

## A new *Drosophila melanogaster* model to identify genetic modifiers of transcriptional repression caused by GAA expansion in *FXN*

Jose V. Llorens[1, 2], Lucia Benito-Jardón[1], Pablo Calap-Quintana[1], Michele Lufino[3], Sirena Soriano[1, 4], Richard Wade-Martins[3], Maria José Martínez-Sebastián[1], Maria Dolores Moltó[1, 5]

*[1] University of Valencia, Spain, [2] University of Uppsala, Sweden, [3] University of Oxford, UK, [4] Baylor College of Medicine, US, [5] Red de Salud Mental, Cibersam, INCLIVA., Spain*

**Background/Hypothesis:** Friedreich ataxia (FRDA), a neurodegenerative disorder with recessive autosomal inheritance, is caused by pathological GAA expansions within the first intron of the *FXN* gene, which leads to a reduction in the level of the encoded protein frataxin. The most accepted hypothesis to explain the transcriptional repression caused by this triplet expansion is the heterochromatinization of the *FXN* locus. However, the underlying molecular mechanism of this process is not completely understood yet.

**Methods:** In order to identify potential factors involved in this process, we developed a new *Drosophila melanogaster* model that consists of two strains expressing the reporter gene firefly luciferase preceded by 9 GAA repeats (normal expansion) in one strain, and 300 GAA repeats (pathological expansion) in the other. To achieve this, three genetic constructs (UAS-Renilla; UAS-9GAA-firefly and UAS-300GAA-firefly luciferases) have been developed based in the pACMAN platform in combination with the UAS-GAL4 system to control the expression.

**Results:** We checked that the 300 GAA repeat expansion represses the firefly luciferase expression, analogously to *FXN* gene repression in FRDA. It was also observed a higher level of chromatin compaction in the luciferase construct of the 300 GAA line compared to the 9 GAA line. Next, we started a genetic screen by crossing both model lines with several *Drosophila* strains carrying alleles of genes involved in posttranslational histone modifications, heterochromatin formation and maintenance, and transcriptional activation or repression. So far, we have identified some potential regulators of the repression mediated by the GAA pathological expansion, as Su(var)3-9, Su(var)2-5 and Su(var)2-1 , which human orthologous are SUV39H and HP1.

**Conclusions:** We have developed a new model in *D. melanogaster* suitable for high-throughput screening to identify specific genetic modifiers involved in transcriptional silencing of *FXN* and thus potential therapeutic targets for the treatment of FRDA.

# Session 3b: Cellular and animal models of other ataxias

## Thursday 26th March

*Chair: Michel Koenig (Institut Universitaire de Recherche Clinique, Montpellier, France)*

Invited speaker

14:00 Olaf Riess (University of Tubingen, Germany)
*Deciphering the pathogenesis of SCA3 using animal models*

Selected presentations

14:30 Olga Baron (King's College London, UK):
*Relevance of autophagy in neurodegeneration in DRPLA*

14:45 Cecilia Mancini (University of Torino, Italy):
*SCA28-Knockin mouse model: severe impairment of mitochondrial fission/fusion network in MEF cells*

15:00 Isabel Onofre (Center for Neuroscience and Cell Biology, Coimbra, Portugal):
*Characterization of a human Machado-Joseph disease neuronal cell model derived from Induced Pluripotent Stem Cells*

15:15 Liliana Santos (University of Minho, Braga, Portugal):
*Determinants of neuron-specific pathogenesis in Machado-Joseph Disease: study in a C. elegans model*

15:30 Natascia Ventura (Leibniz Institute for Environmental Medicine and the Heinrich Heine University of Duesseldorf, Germany):
*In vivo phenotypic-based screening to identify suppressors of mitochondrial-associated ataxias*

15:45 Close of session

16:15 Coaches leave for Oxford

17:15-19:15 Free time

19:15-19:45 Drinks reception at Oxford Town Hall

19:45 Dinner in Oxford Town Hall
There will be presentations by Jeffrey Sherman (Horizon Pharma) who are sponsoring this event and Kyle Bryant (FARA) who has Friedreich's ataxia and is the Founder and Director of rideATAXIA.

Invited Speaker: Olaf Riess (University of Tubingen, Germany)

## Deciphering the pathogenesis of SCA3 using animal models

Spinocerebellar ataxia type 3 (SCA3) is the most frequent autosomal dominant inherited type of ataxias. Pathogenetically, it belongs to the group of polyglutamine repeat disorders such as Huntington's disease or DRPLA. As in these diseases, abnormal protein aggregates are being found in the nucleus of neuronal cells. Generating mouse models we confirmed that also mouse neurons are vulnerable to polyglutamine repeat expansions. Furthermore, we generated inducible models using the Tet-Off system to turn off the expression of the pathogenic transgene. When the transgene expression is turned off in early disease stages, we can reverse the phenotype. We also generate mice in which the aberrant protein is expressed in the nucleus causing a dramatic phenotype whereas the same transgene expressed in the cytoplasm of neurons is only associated with a mild and late onset. We tried to decipher the cause of the nuclear localization of the aberrant protein which is otherwise in the cytoplasm in its wildtype conformation. We detected two strong nuclear export signal in the N-terminus of the protein, and a weak nuclear import signal in the C-terminal region close to the polyglutamine stretch. We proposed that these signal motifs are being separated in the aberrant protein by proteolytic cleavage. Subsequently we crossed mice deficient for calpastatin, the only known natural inhibitor of Ca-induced proteases, calpains, into the transgenic SCA3 mice causing a more severe phenotype confirming our hypothesis of altered protein cleavage of aberrant ataxin3. We next studied the role of the N-terminal cleaved protein in gene trap mice with a fragment mimicking the main calpain cleavage site of ataxin3. Whereas these mice appear to be normal until the age of one year, they die quickly afterwards concluding that the overall pathology in SCA3 is a combination of intranuclear and cytoplasmic pathogenic events. These data support a hypothesis that inhibition of altered protein cleavage might be a therapeutic effective approach in SCA3.

## Relavance of autophagy in neurodegenration in DRPLA

Olga Baron, Ivan Rattray, Gillian Bates, Manolis Fanto

*King's College London, London, United Kingdom*

Dentatorubral pallidoluysian atrophy (DRPLA) is caused by polyglutamine strech in Atrophin 1 gene, a transcriptional cofactor. We showed previously in Drosophila fly model that ectopical expression of human atrophin-1 bearing a 65Q expansion results in neuronal degeneration due to autophagic block. Autophagic disfunction was associated with downregulation of fat gene expression, which is involved in Hippo tumorsupressor signalling pathway. Autophagy is a major cellular degradation system important for cellular homeostasis and disturbances are linked to several neurodegenerative conditions. Exploration whether and how autophagy participates in pathophysiology of DRPLA and the involvement of Ft/Hippo may provide targets for therapeutic approaches.

To confirm the observations made in flies in mammalian system, we are using previously described DRPLA mouse model including the pathologic strain Atn1-65Q and control Atn1-26Q line crossed to a transgenic autophagy reporter line expressing GFP::LC3. Our preliminary data indicates previously described motoric disfunction in Atn1-65Q line, showing deficits in rotarod performance and altered activity. Morphologically, we were able to confirm intranuclear polyglutamine positive inclusions in the Atn1-65Q line.

Regarding autophagy related phenotype, preliminary evaluation of p62 and Lamp2 stainings in 3 weeks old animals show no obvious change in lysosomal Lamp2 positive vesicles, but p62 positive aggregation occurred in several brain regions of Atn1-65Q animals, whereas no or minimal aggregation was evident in wild type animals. Interestingly, we also observed increased accumulation of lipofuscin (aging autopigments, associated with lysosomal storage disorders) in end stage Atn1-65Q mutants if compared to Atn1-26Q and wild type animals. These preliminary results indicate indeed possible autophagy disregulation in mouse model for DRPLA and encourage further investigation of p62 aggregation and autophagosome/lysosome in mice and in DRPLA patient samples using combined methods of immunohistochemistry and western blot assay.
Further, qPCR analysis of expression of mammalian fat orthologs revealed, that while there are no changes at the asymptomatic time point (3 weeks), the expression of fat3 and fat4 are significantly increased in Atn1-26Q and significantly reduced in Atn1-65Q cerebelli of 10 weeks old animals. This resembles the findings previously published in Drosophila model for DRPLA, where the Drosopfila fat was elevated in atrophin overexpressing mutants and reduced in atrophin-polyQ mutants. Further studies on Fat-Hippo pathway involvement in autophagy regulation in DRPLA are very encouraging. Interestingly, we observed a massive reactive astrocyte invasion in Atn1-65Q end stage animals, compared to the controls, which is mainly present in the fiber rich regions of the forebrain tissue. The relevance of this astrocyte activation will be further investigated. This finding is interesting in the light of previous observations in Drosophila, where introduction of the Atrohin-PolyQ in glial cells resulted in neuronal degeneration suggesting a non-autonomous effect due to glia-neuron interaction.

## SCA28-Knockin mouse model: severe impairment of mitochondrial fission/fusion network in MEF cells

Cecilia Mancini[1], Eriola Hoxha[2], Elisa Giorgio[1], Simona Cavalieri[3], Eleonora Di Gregorio[3], Fiorella Altruda[4], Emilia Turco[4], Filippo Tempia[2], Alfredo Brusco[1, 3]

*[1] Department of Medical Sciences, University of Torino, Italy, [2] Neuroscience Institute Cavalieri Ottolenghi (NICO), University of Torino, Italy, [3] Medical Genetics Unit, "Città della Salute e della Scienza" Hospital, Italy, [4] Department of Molecular Biotechnology and Health Sciences, University of Torino, Italy*

SCA28 is one of the 31 known subtypes of autosomal dominant Spinocerebellar Ataxias (SCA) and it is caused by mutations in the AFG3L2 gene, encoding for an ATP-dependent metalloprotease belonging to the AAA-superfamily (ATPases Associated with a variety of cellular Activities). AFG3L2 can form homo- or hetero-oligomeric complexes with paraplegin: both reside in Inner Mitochondrial Membrane (IMM) and exert protein quality surveillance and mediate protein processing. SCA28 patients mainly have missense mutations in the peptidase domain of AFG3L2, but the function altered by the mutations is still unclear. To further evaluate the role of missense changes, we generated a knockin (KI) mouse model carrying the p.M665R mutation (human p.M666R, showing the most severe phenotype). It should be noted that two SCA28 mouse models are described in literature, but both carry a loss-of-function mutation in Afg3l2. SCA28 M665R-KI heterozygous mice showed a phenotype onset between 16 and 18 months, while homozygous M665R gave a perinatal lethality. We studied mitochondrial dynamics in Mouse Embryonic Fibroblasts (MEFs) from KI and WT animals: (i) AFG3L2 protein levels were comparable; (ii) OPA1, a protein found in the inner mitochondria membrane, involved in mitochondria fusion process, showed an increase of the short forms and absence of the long forms; (iii) mitochondrial network morphology by mitoRED staining suggested a complete fragmented pathway in homozygous KI and an intermediate tubular/fragmented network in heterozygous MEFs. Taken together, these data show an impairment of OPA1 processing that results in increased mitochondrial fragmentation. This is in accordance with the cellular phenotype seen in Afg3l2 knockout mouse model, corroborating the idea that SCA28 mutations hitting the peptidase domains negatively impact on m-AAA complex function.

## Characterization of a human Machado-Joseph disease neuronal cell model derived from Induced Pluripotent Stem Cells

Isabel Onofre[1, 2], Nuno Mendonça[3], Cristina Januário[3], Niels Geijsen[4], Luis Pereira de Almeida[1, 2]

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Spinocerebellar ataxia type 3 (SCA3) also known as Machado Joseph Disease (MJD) is an autosomal dominant inherited cerebellar ataxia and a progressive, adult-onset neurodegenerative disease. MJD is caused by a CAG-repeat expansion in the ATXN3 gene on chromosome 14q24.3–q32.2, which results in an abnormally long polyglutamine tract in the ataxin-3 protein. With the development of induced pluripotent stem cell (iPS) cell technology, the study of the pathology of MJD is no longer restricted to artificial disease modeling systems such as animal models or cell lines, which present limitations as models of human neurogenetic disorders.

Therefore, the aim of this work was to produce a novel human cell model of Machado-Joseph disease, which is expected to complement other disease models and bring further insight on disease mechanisms and pathology. We derived disease-specific iPS cell lines from MJD patient's fibroblasts, through the induced expression of the four transcription factors OCT3/4, SOX2, KLF4 and c-MYC using a lentiviral vector. Several clones were selected after characterization based on positive pluripotent cell surface markers (SSEA4, TRA1-80), endogenous levels of pluripotency related genes (NANOG, OCT4, SOX2, REX1, DNMT3B, ABCG2) and in vitro and in vivo differentiation potential. Neural induction and neuronalization of these cultures was performed using a specific set of morphogens for hindbrain/midbrain patterning and the iPS-derived neurons were then analyzed for both terminal maturation and functional markers. Presently, we are evaluating morphological alterations, such as the number of neurites and neurite arborization, toxic protein clearance, $Ca^{2+}$ dependent proteolysis of ATXN3 via excitotoxic glutamate-induced $Ca^{2+}$ influx and susceptibility to oxidative stress.

Our final goal is the validation of this new in vitro model as a robust disease modeling system, recapitulating the molecular and cellular phenotypes typical of MJD and opening new opportunities for investigating the disease pathogenesis and for drug screening.

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## Determinants of neuron-specific pathogenesis in Machado-Joseph Disease: study in a *C. elegans* model

Liliana Santos[1, 2], Stéphanie Oliveira[1, 2], Andreia Teixeira-Castro[1, 2], Patrícia Maciel[1, 2]

*[1] Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Portugal, [2] ICVS/3B's - PT Government Associate Laboratory, Portugal*

Machado-Joseph Disease (MJD) is a late-onset neurodegenerative disorder, caused by a CAG triplet expansion within the ATXN3 gene. The mutant ataxin-3 protein (ATXN3) shows a strong tendency to misfold and aggregate leading to the formation of neuronal inclusions in specific brain regions, the hallmarks of pathology. Knowing that both WT and mutant ATXN3 are ubiquitously expressed throughout the brain and differences in ATXN3 aggregation fail to correlate with ATXN3 tissue/neuron-specific expression levels or heterogeneity of polyQ-length, we posed a challenging question: what causes the neuron-specific pattern of degeneration underlying MJD? To address this question we categorized the 302 C. elegans neurons regarding their function and neurochemical content and mapped the neurons showing increased susceptibility to mutant ATXN3 expression in a transgenic C. elegans model for MJD pathogenesis. Available fluorescent neuronal markers that specifically label distinct neuron subtypes allowed aggregation scoring. In vivo confocal analysis allowed classification of neurons as susceptible to mutant ATXN3 expression when ATXN3 aggregates co-localized with the neuronal marker within a single confocal slide. We show that GABAergic and Cholinergic motor neurons localized in the head are severely affected by mutant ATXN3 aggregation while those localized in the ventral nerve cord are mildly affected. Serotonergic pharyngeal neurosecretory-motor neuron cell body contained mutant ATXN3 aggregates in ~80% of the analyzed animals, contrasting with the serotonergic hermaphrodite-specific neuron cell bodies that never present aggregation. Some glutamatergic chemosensory and thermosensory neurons are susceptible to mutant ATXN3 expression, while all dopaminergic neuronal cell bodies seem highly resistant. So far we found no evidences of neuronal cell death in our disease model, as assessed by TUNEL and SYTO dye assays – in spite of the marked neuronal dysfunction. The results obtained so far strongly suggest that mutant ATXN3 aggregation in C. elegans neurons is not stochastic but neuronal-subtype specific and that the neuronal proteostasis environment in which aggregation-prone proteins are expressed determine its aggregation state. Based on these results we are now addressing the transcriptomic signature of our MJD model nervous system aiming to identify the molecular basis underlying the differential susceptibility.

## *In vivo* phenotypic-based screening to identify suppressors of mitochondrial-associated ataxias

Natascia Ventura[1, 2], Silvia Maglioni[1, 2], Alfonso Schiavi[2]

*[1] Institute of Clinical Chemistry and Laboratory Diagnostic Medical Faculty, Heinrich Heine University, Germany, [2] Leibniz Research Institute for Environmental Medicine, Germany*

The use of model organisms for *in vivo* screening is exponentially growing in the last decade. The nematode *Caenorhabditis elegans* is a powerful genetic tractable organism largely employed to gain insight into biological processes and to model human diseases. One third of *C. elegans* cells are organized in a simple yet well-characterized nervous system, making it an elective model organism to study neurodegenerative disorders and to screen for neuroprotective interventions: all mammalian neuronal types are represented, fluorescent tagging allows observation of structural changes of every neuron throughout animal lifetime, and several behavioral assays exist to address the functions of specific neurons.

Different *C. elegans* models for neurodegenerative diseases (e.g. Parkinson, Alzheimer) have been therefore established so far and yielded important insight on dieseases pathogenesis and possible targeted therapeutic interventions. However, most genes responsible for mitochondrial-associated diseases are pleiotropic and non redundant and their complete deficiency is often lethal. To overcome the lethality problem, and to better mimic the human diseases due to reduced expression rather than complete protein deficiency, we progressively decreased the expression of different mitochondrial-associated ataxias genes in *C. elegans* (e.g. frataxin/f*rh-1*, paraplegin/*spg-7*) through modulation of the RNA-interference potency. Thus, we developed and characterized different ataxia diseases models such as Friedreich's ataxia or SCA (Ventura et al., 2005; Ventura and Rea 2007; Maglioni et al. *in process*). Such approach revealed distinct phenotypic and biochemical changes associated with different levels of mitochondrial dysfunction: While severe deficiency of different mitochondrial proteins induces deleterious effects (arrest development, severe neuronal dysfunction), their moderate suppression is associated with phenotypes (smaller but healthier and longer-lived animals), which denote the activation of beneficial compensatory pathways (Rea, Ventura and Johnson, 2007; Schiavi et al. 2013; Maglioni et al. 2014).

The very reproducible and distinct phenotypic changes associated with the different levels of mitochondrial stress represent ideal parameters for automated microscopy quantification, and allowed us to develop a phenotypic-based workflow to screen for genetic or pharmacological modifiers. In the past we could indeed genetically manipulate the different phenotypes (Ventura et al. 2009; Schiavi et al. 2013), and this, coupled with the high homology between nematode and human genes, and with the utilization of FDA approved drugs and food factors, support the rationale of using our newly developed ataxias models to discover novel potential targeted interventions for translational treatments of the corresponding human diseases.


**Pfizer's Rare Disease Research Unit is proud to sponsor the 2015 International Ataxia Research Conference in support of individuals and families with ataxias.**

# Session 4: Cellular and systemic pathways

## Friday 27th March

*Chairs: Giovanni Manfredi (Cornell University, USA) and Henry Paulson (University of Michigan, USA)*

Invited speakers

8:30 Kamran Khodakhah (Albert Einstein College of Medicine, USA):
*Aberrant cerebellar output in ataxia – a common theme*

9:00 Henry Paulson (University of Michigan, USA):
*Why does polyglutamine expansion cause dominantly inherited ataxia? Lessons from SCA3*

Selected presentations

9:30 David Alsina (Universitat de Lleida, Spain):
*Yeast Flavohemoglobin (YHB1) and Nitric Oxide, new players in frataxin deficient yeast*

9:50 Javier Diaz-Nido (Universidad Autonoma de Madrid, Spain):
*DNA repair deficit and neuroinflammation as potential contributors to the physiopathology of Friedreich's ataxia*

10:10 Break

10:40 Sofia Esteves (Life and Health Sciences Research Institute (ICVS), Portugal):
*Citalopram treatment ameliorates motor impairment and suppresses ataxin-3 aggregation in a Machado-Joseph disease mouse model*

11:00 Angelical Martin (Duke University, USA):
*The role of acetylation in the pathogenesis of Friedreich's ataxia*

11:20 Joaquim Ros (Universitat de Lleida, Spain):
*Viability of frataxin-deficient dorsal root ganglia neurons is recovered by calcium chelators and mitochondrial pore inhibitors*

11:40 Amanda Stram (Indiana University School of Medicine, USA):
*Mitochondrial protein hyperacetylation is associated with early diastolic dysfunction in a model of Friedreich's ataxia hypertrophic cardiomyopathy*

12:00 Close of session

Invited Speaker: Kamran Khodakhah (Albert Einstein College of Medicine, US)

## Aberrant cerebellar output in ataxia – a common theme

Esra Tara, Ambika Tewari, Kamran Khodakhah

*Albert Einstein College of Medicine, US*

To affect motor function cerebellar ataxia are likely associated with cerebellar outputs that fail to provide the information necessary for motor coordination. The factors that result in loss of information are diverse, from degeneration of cerebellar Purkinje cells to their abnormal pacemaking and altered synaptic transmission. Yet the final common hallmark of dysfunction of the computational circuitry of the cerebellum in many cerebellar ataxias appears to be aberrant cerebellar output such that the signal to noise ratio of the cerebellar information content is significantly reduced. The findings in several cerebellar mouse models will be discussed together with potential therapeutic interventions.

Invited Speaker: Henry Paulson (University of Michigan, US)

## Why does polyglutamine expansion cause dominantly inherited ataxia? Lessons from SCA3

The events contributing to disease pathogenesis in the dominantly inherited Spinocerebellar Ataxias (SCAs) are complex and only partly elucidated. Polyglutamine expansion in disparate genes is the most common cause of SCA. Yet despite this shared mutational mechanism, much remains unknown about how expansion of a polyglutamine stretch within distinct proteins causes neuronal dysfunction and ultimately neuronal cell death. Moreover, the basis of selective neuronal loss in the various polyglutamine SCAs remains uncertain. Focusing primarily on SCA3 and its disease protein ATXN3, I will discuss the factors contributing to neuronal dysfunction and neuronal cell loss in the polyglutamine ataxias, including protein context of the expansion, alternative splicing of disease genes, protein misfolding and aggregation, transcriptional dysregulation, and altered electrophysiological properties induced by the disease protein. I will conclude by discussing possible routes to therapy based on emerging insights into disease pathogenesis.

## Yeast Flavohemoglobin (YHB1) and Nitric Oxide, new players in frataxin deficient yeast

David Alsina, Joaquim Ros, Jordi Tamarit

*Departament de Ciències Mèdiques Bàsiques, Universitat de Lleida, IRB Lleida, Spain*

Frataxin deficient yeasts have been a very useful tool to analyze frataxin functions and phenotypes caused by frataxin deficiency. Previously, we developed a yeast conditional mutant in which the YFH1 gene (from Yeast Frataxin Homologue 1) was placed under the control of a tet promoter. This model allowed us to analyze chronologically the different phenotypes caused by Yfh1 deficiency. Induction of the iron regulon and oxidative stress were classified as primary events, whereas, loss of ISC proteins was classified as a secondary event caused by the activation of CTH2, a gene of the iron regulon (Moreno-Cermeño, Alsina et al., BBA-MCR 2013). In a transcriptomic and proteomic analysis of these mutants, we found an induction in Yeast Flavohemoglobin 1 (YHB1) early after frataxin repression. This protein is known to interact with frataxin, it has mitochondrial and cytosolic localization, it has nitric oxide (NO) oxidorreductase activity, and its expression can be induced by NO and reactive oxygen species.

In order to analyze the relationship between this protein and frataxin, we constructed double mutant $tetO_2$YFH1 Δyhb1 and a GFP tagged version of YHB1 in a $tetO_2$YFH1 background. Using these mutants we confirmed the increase of YHB1 after YFH1 deficiency at mRNA and protein level. YHB1-GFP was detected in both mitochondria and cytosol (as previously reported by other authors) and its localization was not altered after YFH1 depletion. Interestingly, we found that iron accumulation caused by Yfh1 depletion was prevented in YHB1 mutants, suggesting that this protein is involved in the activation of the iron regulon in YFH1 mutants. Finally, we found an early increase in NO levels in YFH1 deficient mutants. This increase could explain the induction of YHB1 after Yfh1 depletion and suggests a relationship between frataxin deficiency and NO. In summary, the presented results indicate the existence of an interplay between frataxin, Yhb1, NO and iron that could be crucial to understand the early consequences of frataxin deficiency on yeast cells.

## DNA Repair Deficit and Neuroinflammation as Potential Contributors to the Physiopathology of Friedreich's Ataxia

Jara Moreno-Lorite[1, 2, 3], Frida Loria[1, 2, 3], Sara Perez-Luz[1, 2, 3], Daniel Oberdoerfer[1, 2, 3], Yurika Katsu-Jiménez[1, 2, 3], Oscar Yang[1, 2, 3], Javier Diaz-Nido[1, 2, 3]

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Friedreich's ataxia (FA) is a recessive and predominantly neurodegenerative disorder caused by a decreased level of frataxin protein. To gain some insight into the molecular mechanisms contributing to neurodegeneration in FA we have studied human neural cell models subjected to frataxin knockdown.

We have obtained an inducible neuron-like cell model for frataxin deficiency by stable transduction of the human neuroblastoma SH-SY5Y cell line with a tetracycline-inducible lentiviral vector encoding for a specific shRNA. Enhanced oxidative stress, DNA damage and activation of apoptotic cell death was observed upon frataxin knockdown in this model. Interestingly, frataxin down-regulation was also accompanied by significant changes in the expression of various proteins implicated in DNA repair. These changes were reversible after up-regulation of frataxin gene expression. In view of these data we suggest that increased DNA damage in frataxin-deficient neuronal cells may be due not only to oxidative stress but also to diminished DNA repair systems.

In order to study the contribution of glial cells to the physiopathology of FA, we have analyzed the consequences of frataxin knockdown in cultured human astrocytes, which also results in increased oxidative stress and apoptotic cell death. Interestingly, frataxin silencing in astrocytes is also accompanied by an enhanced expression and secretion of some pro-inflammatory cytokines.

To test for non-cell autonomous interactions we cultured wild-type mouse neurons in the presence of frataxin-deficient astrocyte conditioned medium, which provoked a delay in the maturation of these neurons, a decrease in neurite length and enhanced cell death. These findings indicate a detrimental effect of frataxin silencing, not only for astrocytes but also for neuron-glia interactions, underlining the need to take into account the role of non cell-autonomous processes in the pathogenesis of FA.

Furthermore, our studies performed with cultured olfactory mucosa stem cells, which are obtained from biopsies from FA patients, also indicate a deficiency in DNA repair-related proteins as well as an increased expression and secretion of some pro-inflammatory cytokines. These results support the view that both DNA repair deficit and neuroinflammation may be potential contributors to the physiopathology of FA.

*This research is supported by Spanish MINECO, CIBERER, Comunidad Autonoma de Madrid and AFAF (Association Française de l'Ataxie de Friedreich).*

## Citalopram treatment ameliorates motor impairment and suppresses ataxin-3 aggregation in a Machado-Joseph disease mouse model

Sofia Esteves[1, 2], Sara Duarte-Silva[1, 2], Anabela Silva-Fernandes[1, 2], Andreia Teixeira-Castro[1, 2], Patrícia Maciel[1, 2]

*[1] Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Portugal, [2] ICVS/3B's - PT Government Associate Laboratory, Portugal*

Machado-Joseph disease (MJD) or Spinocerebellar ataxia type 3 (SCA3) is a late-onset autosomal dominant neurodegenerative disorder caused by the expansion of a polyglutamine tract in the C-terminus of the ATXN3 gene product, ataxin-3. MJD causes progressive cerebellar ataxia, which results in a lack of muscle control and coordination. So far, there is no treatment available for MJD. Citalopram, belonging to the Selective Serotonine Reuptake Inhibitors class, was identified in an unbiased screen of FDA-approved compounds, as markedly reducing motor impairment and ataxin-3 aggregation in C. elegans. Chronic citalopram treatment with two dosages (8mg/kg and 13mg/kg) in our CMVMJD135 mouse model rescued the reduction in body weight gain and improved gait, tremors, and limb clasping mainly with the lowest dosage. Motor balance and coordination defects were strikingly ameliorated through the balance beam walk, motor swimming and footprinting tests. At the pathological level, citalopram treatment rescued astrogliosis in the substantia nigra and suppressed ataxin-3 neuronal inclusions in affected brain regions (pontine nuclei, reticulotegmental nucleus of the pons, facial nuclei and lateral reticular nuclei), with no impact in total ataxin-3 protein levels. The observation of a direct impact on ATXN3 protein soluble state suggests an improvement of cell proteostasis, which does not seem to be related to ATXN3 degradation, as total steady-state levels of the mutant protein remain unchanged. We are trying to unravel the mechanisms through which citalopram induces this protective response. Globally, our results suggest serotonergic signaling as a promising therapeutic target for the delay of MJD progression.

## The role of acetylation in the pathogenesis of Friedreich's ataxia

Angelical, S. Martin[1], Gregory, R. Wagner[1, 2], Kathleen, A. Hershberger[1], R. Mark Payne[2], Matthew, D. Hirschey[1]

*[1] Duke University, US, [2] Indiana University, US*

**Background/Hypothesis:** The heart's ability to adaptively use metabolic substrates to drive energy production—known as energetic substrate flexibility—must be maintained, as even subtle variations in efficiency have severe impacts on cellular metabolic health. Lysine acetylation and its regulation by the mitochondrial $NAD^+$-dependent protein deacetylase sirtuin 3 (SIRT3) are emerging as important regulators of cardiac energy homeostasis. Reduction in SIRT3 activity in the heart results in hyperacetylation of metabolic enzymes, hypertrophy, and markedly reduced ATP levels (>50%)—demonstrating an important role for acetylation in regulating cardio-bioenergetics. In the well-established cardiac mouse model of Friedreich's Ataxia (MCK-FA), mice experience progressive, reversible hyperacetylation of mitochondrial proteins, implicating a role for acetylation and SIRT3 activity in mediating energy homeostasis in FA hearts. Taken together, we predict that hyperacetylation of cardiac metabolic proteins contributes to the impaired, gradual decline in substrate flexibility and ultimate cardiac failure in FA.

**Methods:** To determine the role of acetylation in the pathogenesis of FA, we aim to modulate acetylation status and SIRT3 activity in MCK-FA mice using three parallel strategies: (1) dietary supplementation with NAD+ precursors to boost sirtuin activity, (2) genetic manipulation of SIRT3 to modulate expression and (3) manipulation of metabolic substrate use to control oxidation and subsequent acetylation. We will monitor changes in cardiac function, substrate usage and bioenergetics to examine a role of SIRT3 and acetylation in regulating energy homeostasis in FA.

**Results/Conclusions:** Prior studies show that $NAD^+$ and its precursors can reduce cardiac hyperacetylation in mouse models and protect animals against hypertrophy in a SIRT3-dependent manner. First, using $NAD^+$ precursor nicotinamide mononucleotide (NMN), we boosted $NAD^+$ levels and reduced mitochondrial acetylation (-1.43 fold) in the MCK-FA heart. Further functional studies are underway to assess changes in SIRT3 activity with NMN supplementation. Second, we are collaborating with R. Mark Payne, MD to generate SIRT3 knockout or overexpression MCK-FA mice to directly test the function of SIRT3 activity on energy metabolism; data will be presented separately. Third, we used transcriptomic, metabolomic, proteomic and other analyses to explore a role for acetylation in contributing to the compromised substrate flexibility of the MCK-FA heart. Our studies in late stage MCK-FA animals have thus far revealed transcriptional downregulation of pathways involved in catabolism of fatty acids, ketones and amino acids. These transcriptional data are further supported by metabolomic analyses. Furthermore, functional assays reveal significantly reduced oxidation of fatty acids and ketones in the MCK-FA heart (-1.64 and -1.67 fold, respectively). We are currently conducting temporal biochemical utilization studies to further understand and ultimately manipulate the metabolism of substrates that may most contribute to the progressive hyperacetylation. Overall, this work allows us to explore altering protein acetylation as a therapeutic strategy to modulate mitochondrial energy homeostasis in FA hearts.

## Viability of frataxin-deficient dorsal root ganglia neurons is recovered by calcium chelators and mitochondrial pore inhibitors

Joaquim Ros, Stefka Mincheva, Marta Llovera, Jordi Tamarit

*Departament de Ciències Mèdiques Bàsiques, IRB-Lleida, Universitat de Lleida, Spain*

To understand the cellular consequences of frataxin deficiency we use primary cultures of dorsal root ganglia (DRG) neurons as cell model because this tissue is primarily affected in the disease. Reduction of 80% of frataxin levels in these cells was achieved by transduction with lentivirus containing shRNA silencing sequences. These frataxin-deficient cells show neurite degeneration and apoptotic cell death. Phosphorylated neurofilament NF-200, cleavage of caspase 3 and increased levels of Bax and phosphorylated CREB are, among others, markers observed in these cells. A significant increase of free intracellular Ca2+ levels and alteration in Ca2+-mediated signaling pathways was also observed; in this context, the activation of calpain was observed by cleavage of one of its substrates, α-fodrin; such cleavage can be avoided by BAPTA, an intracellular calcium chelator. These results suggest that altered calcium homeostasis can play a pivotal role in neurodegeneration caused by frataxin deficiency. These features can be reversed with the addition of a cell-penetrant TAT peptide coupled to the BH4 anti-apoptotic domain of Bcl-xL protein. Additionally, frataxin depletion caused mitochondrial membrane potential decrease. We have recently observed that after frataxin depletion, a marked increase in cyclophilin D, a protein involved in opening the mitochondrial permeability transition pore, occurs. In an attempt to avoid toxic effects caused by low frataxin levels, we treat the cultures with cyclosporin A, a cyclophilin D inhibitor. Preliminary results indicate that survival is recovered to a significant extent. Other compounds are also currently tested in DRG neuron cultures with the aim of decreasing the deleterious impact of frataxin reduction on cell physiology.

As a conclusion, the use of this cell model provide precise clues to understand the physiological events taking place after frataxin depletion and the rationale for new therapies.

## Mitochondrial protein hyperacetylation is associated with early diastolic dysfunction in a model of Friedreich's ataxia hypertrophic cardiomyopathy

Amanda R Stram[1], Gregory R Wagner[2], Melanie P Pride[1], Steven Messina-Graham[1], Hal Broxmeyer[1], Matthew D Hirschey[2], R Mark Payne[1]

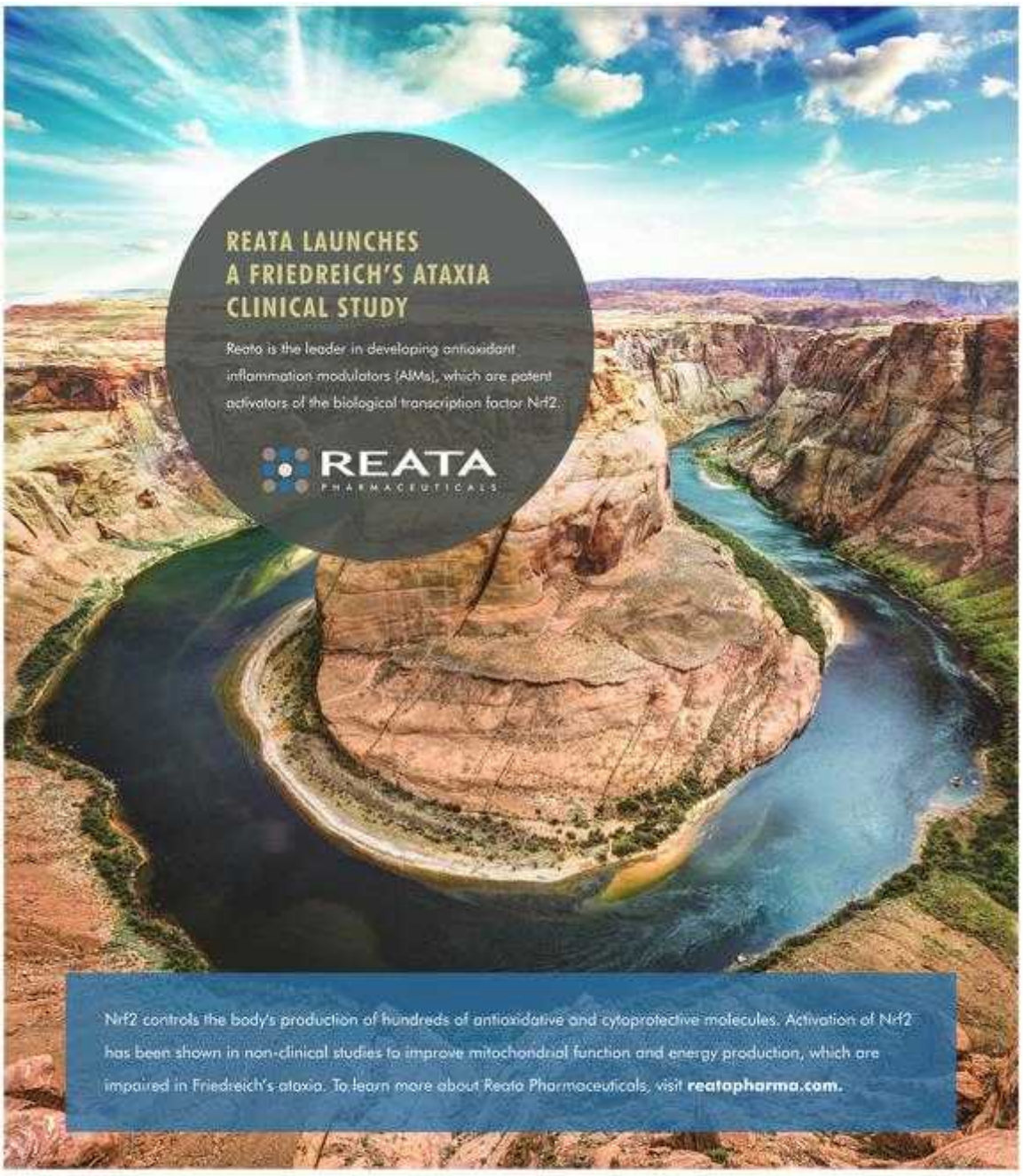
*[1] Indiana University School of Medicine, US, [2] Duke University, US*

**Background**: We hypothesized that mitochondrial protein hyperacetylation is associated with diastolic dysfunction in Friedreich's Ataxia (FRDA) cardiomyopathy. We had reported that Frataxin (FXN) loss results in decreased activity of the mitochondrial deacetylase, sirtuin 3 (SIRT3), and cardiac mitochondrial protein hyperacetylation. SIRT3 targets enzymes important to energy homeostasis, suggesting hyperacetylation contributes to metabolic derangement in FRDA.

**Methods**: A conditional mouse model with ablation of FXN in heart and skeletal muscle (FXN MCK-Cre KO, or "FXN KO") was compared to controls at postnatal days 30, 45 and 65. Heart function was measured using echocardiogram and cardiac catheterization. Heart lysate was probed for lysine acetylation. Myocardial histology was performed using Masson's Trichrome and electron microscopy. Respiration of isolated cardiac mitochondria was measured with a Seahorse analyzer.

**Results**: FXN KO hearts show age-progressive mitochondrial hyperacetylation associated with a slower rate of oxidative phosphorylation ($p < 0.01$). Electron microscopy demonstrates abnormal mitochondrial morphology as early as day 30 in FXN KO mice with loss of cristae content, and disorganized dysmorphic mitochondria by day 65. Histology demonstrates increased myocardial fibrosis. Diastolic dysfunction is evident by day 45 (n=8), with FXN KO mice having LVH ($p<0.01$), and increased mitral E/A ratio vs controls ($p<0.01$), yet no difference in systolic parameters. At day 65 (n=8), diastolic dysfunction in FXN KO is apparent by increased mitral E/A ($p<0.01$), tissue Doppler E/E' ($p<0.01$), IVRT, and Tau (τ) ($p<0.01$), and decreased -dP/dt ($p<0.001$). Systolic failure is evident in FXN KO at day 65 (n=8) with reduced EF, FS, +dP/dt ($p<0.001$), and ESPVR ($p<0.01$). To explore the role of SIRT3, we generated SIRT3-FXN KO mice, with loss of both SIRT3 and FXN cardiac expression ("double KO"). Double KO mice demonstrate a more severe functional cardiac phenotype compared to FXN KO, primarily in systolic parameters (N=2), with decreased EF, FS and SV ($p<0.05$). Left ventricular mass and wall thickness were no different between groups. Interestingly, the double KOs were more susceptible to stress (surgery and echo). The double KO (N=8) developed obesity with higher average body weight compared to FXN KO (24.9g vs 21.4g, $p<0.01$). Heart weight was unchanged, leading to statistically higher heart:body weight ratio in the FXN KO ($p=0.02$).

**Conclusions**: Mitochondrial protein hyperacetylation is associated with abnormal mitochondrial function and early diastolic dysfunction in a mouse model of FRDA hypertrophic cardiomyopathy. Loss of expression of both SIRT3 and FXN results in a more severe cardiac phenotype and obesity. This may reflect impairment of the normal post translational regulation of metabolic proteins by SIRT3. We are currently investigating the relationship between SIRT3 activity, expression, and acetylation, and its impact on heart function, which will provide important insight into the pathophysiology of FRDA cardiomyopathy.




**Clinical Study on RTA 408 Capsules**

Reata Pharmaceuticals is actively enrolling the MOXIe study, a placebo-controlled, multicenter clinical study of RTA 408 capsules in Friedreich's ataxia. For more information, go to **clinicaltrials.gov.**

MOXIe
*a study in Friedreich's ataxia*

# Session 5: Drug discovery & emerging therapeutic strategies

## Friday 27th March

*Chairs: Roberto Testi (University of Rome Tor Vergata, Italy) and Rob Wilson (University of Pennsylvania, USA)*

14:00 Memorial to the late Earl Giller to whom this session is dedicated – Ron Bartek (FARA)

Invited speaker

14:05 Joel Gottesfeld (Scripps research Institute, USA):
*Mechanism of action of 2-aminobenzamide HDAC inhibitors in reversing gene silencing in Friedreich's ataxia*

Selected presentations

14:35 Hagar Greif (Bioblast Pharma Ltd., Tel Aviv, Israel):
*BB-FA (TAT-MTS(cs)-Frataxin) exhibits promising potential as a protein replacement drug candidate for Friedreich's Ataxia*

14:55 Kevin Kemp (University of Bristol, UK):
*The neuroprotective and neuroregenerative properties of bone marrow stem cell mobilising drugs in Friedreich's ataxia*

15:15 Fatih Ozsolak (RaNA Therapeutics, Cambridge, US):
*Stabilization of FXN mRNA using oligonucleotides for the treatment of Friedreich's ataxia*

15:35 Break

15:55 Giorgio Casari (San Raffaele Scientific Institute, Milan, Italy):
*Genetic and pharmacological rescues of spinocerebellar ataxia in the SCA28 model open to human therapy*

16:15 Luis Pereira de Almeida (University of Coimbra, Coimbra, Portugal):
*Transplantation of cerebellar neural stem cells alleviates motor coordination and neuropathological deficits of a transgenic mouse model of Machado-Joseph disease*

16:35 Alessandra Rufini (University of Rome "Tor Vergata, Italy):
*Therapeutic strategies to prevent the ubiquitin/proteasome-dependent degradation of frataxin*

16:55 Jacques P. Tremblay (Laval University, Quebec, Canada):
*An AAV9 coding for frataxin clearly improved the symptoms and prolonged the life of Friedreich ataxia mouse models*

17:15 Joana Duarte-Nueves (Center for Neuroscience and Cell Biology, University of Coimbra, Portugal):
*Therapeutic role of Neuropeptide Y in mouse models of Machado-Joseph Disease*

*17:35* Florence Malisan (Department of Biomedicine and Prevention, University of Rome "Tor Vergata", Italy):
*Src inhibitors modulate frataxin protein levels*

17:55-18:00 Close of session

This session is dedicated to the late Earl Giller for his valued contribution to this area of research

The FA family lost a dear friend and treasured mentor last April, when Dr. Earl Giller died at age 70 after a long and courageous battle with melanoma. We would like to present in Dr. Giller's honor this next session on Drug Discovery and Emerging Therapeutic Strategies - an area in which Earl's insights, guidance and dedication were essential to our ability to make significant strides toward treating FA.

Earl earned a Bachelor of Science degree from Brown University in 1965 and joint M.D./Ph.D. degrees in Neurochemistry from New York University's School of Medicine in 1971. He taught at the Schools of Medicine of Yale and the University of Connecticut before joining Pfizer in 1992, where he became a global clinical leader in drug development. Later, he was Vice President of Clinical Development at Marinus Pharmaceuticals. He authored over 80 peer-reviewed articles, numerous abstracts and book chapters and edited two books on post-traumatic stress disorder. He served his country at the U.S. Department of Veterans Affairs, as well as at the National Institutes of Health. We are proud, honored and grateful to say that Earl also served on the FARA Board of Directors, Scientific Review Committee and Scientific Advisory Board for over six years.

Though Earl did not have a family connection to FA, he treated the FARA community like family and was whole-heartedly devoted to our effort to treat and cure FA. We are confident we will be successful in that effort and, when we are, it will have been in no small measure due to Earl Giller's extraordinary generosity of time, talent and treasure. We always knew that, when the tough scientific decisions needed to be made, Earl's wisdom, judgment and grace could be counted upon to point the way to consensus around a sound approach. We will remain grateful, too, for the giant strides we have made in the very important partnerships between FARA and our pharmaceutical industry colleagues – essential partnerships that Earl helped inspire, establish and nurture. And, he helped us accomplish all of that with such a gentle presence -- one thoughtful, wise, soft-spoken word at a time.

We will always remember and treasure Earl Giller as a real friend, an inspiring, invaluable colleague, a true gentleman, a remarkable and wonderful man. Please join us, then, in dedicating this session on *Drug discovery and emerging therapeutic strategies* to Dr. Earl Giller.

Invited Speaker: Joel Gottesfeld (Scripps Research Institute, USA)

## Mechanism of action of 2-aminobenzamide HDAC inhibitors in reversing gene silencing in Friedreich's ataxia

Elisabetta Soragni[1], C. James Chou[1,2], James R. Rusche[3], and Joel M. Gottesfeld[1]

*[1]Department of Cell and Molecular Biology, The Scripps Research Institute, US, [2]Medical University of South Carolina, US, [3]Repligen Corporation, US*

Loss of the essential mitochondrial protein frataxin in Friedreich's ataxia is due to heterochromatin-mediated silencing of the nuclear *FXN* gene. While the mechanism whereby expanded GAA•TTC triplet repeats in the first intron of the *FXN* gene induce heterochromatin has not been fully established, histone posttranslational modifications near the repeats and at the *FXN* promoter are fully consistent with an epigenetic silencing mechanism. Our laboratory has generated patient induced pluripotent stem cell (iPSC) lines, and we find that iPSC-derived neuronal cells recapitulate heterochromatin signatures and *FXN* gene silencing first identified in patient lymphoid cells and fibroblasts. Previous studies identified a class of small molecule histone deacetylase (HDAC) inhibitors that increase *FXN* mRNA levels and frataxin protein in patient cells, mouse models and in the FRDA neuronal cells. We find that only 2-aminobenzamide HDAC inhibitors that target the class I HDAC enzymes (HDACs 1 – 3) are active in restoring *FXN* gene expression. Structural analogs of the active HDAC inhibitors that selectively target either HDAC1 or HDAC3 do not show similar increases in *FXN* mRNA levels. Chromatin signatures indicate that histone H3 lysine 9 is a key residue for gene silencing through methylation and reactivation through acetylation, mediated by the HDAC inhibitor. One member of our library of 2-aminobenzamide HDAC inhibitors has been investigated in a Phase Ib clinical trial in FRDA patients. Drug treatment lead to increases in *FXN* mRNA and histone acetylation at the *FXN* gene in peripheral blood mononuclear cells in treated patients. As in the neuronal cells, increases in histone H3 lysine 9 acetylation paralleled increases in *FXN* mRNA. Interestingly, the concentration of drug required to induce epigenetic changes in neuronal cells is comparable to the exposure in patients required to observe increases in histone acetylation and gene activation. While the 2-aminobenzamides are promising therapeutics for FRDA, further development of this compound class will be necessary to identify molecules for chronic use. We have explored the mechanism of action of this compound class and our efforts to identify improved molecules for future clinical study will be summarized. Additionally, by interrogating microarray data from neuronal cells treated with inhibitors of different specificity, we identify two genes encoding histone macroH2A (*H2AFY2*) and Polycomb group ring finger 2 (*PCGF2*) that were specifically down-regulated by the inhibitors targeting HDACs1 and 3 versus the more selective inhibitors. Both genes are involved in transcriptional repression and we speculate their involvement in *FXN* gene silencing. Our results shed light on the mechanism whereby HDAC inhibitors increase *FXN* mRNA levels in FRDA neuronal cells.

## BB-FA (TAT-MTS(cs)-Frataxin) exhibits promising potential as a protein replacement drug candidate for Friedreich's Ataxia

Hagar Greif[1], Haya Lorberboum-Galski[2], Dalia Megiddo[1]

*[1] Bioblast Pharma Ltd., Israel, [2] The Hebrew University Medical School in Hadassah, Israel*

Bioblast Pharma is a clinical stage company focus on developing multiple drugs for rare diseases. Bioblast's mitochondrial Protein Replacement Therapy (mPRT) platform is based on a novel fusion protein, comprised of a delivery moiety that contains TAT (as membrane carrier) and mitochondrial-transport-signal (MTS) that enables cleavage and anchorage in the mitochondria, fused to a therapeutic replacement protein. MTS origin is either homologous (native to the protein) or heterologous (taken from another mitochondrial protein). This platform is currently in preclinical development for two diseases: Friedreich's Ataxia and Ornithine Transcarbamylase Deficiency.

In vitro studies conducted in collaboration with Prof. Haya Lorberboum-Galski (the Hebrew University Medical School in Hadassah, Jerusalem) demonstrated that our unique fusion protein – TAT-heterologous-MTS-FXN – had better bacterial expression, higher cells penetration in patient's cell lines, more efficient internalization into the mitochondria and consequently better mitochondrial activity, as compared to TAT-homologous-MTS-FXN. Indeed, the recombinant fusion protein (BB-FA) containing the human mitochondrial citrate synthase MTS (TAT-MTS(cs)-FXN fusion protein) internalized successfully into the mitochondria and demonstrated increased Aconitase activity in several patient's cell lines.

In animal studies, following biweekly administrations of 100 and 400µg BB-FA (TAT-MTS(cs)-FXN) for 21 days, BB-FA fusion protein was shown to internalized into the mitochondria of FA mice model (FVB;B6-Tg(FXN)1Sars Fxntm1Mkn/J, aka "Sarsero" model). The internalized protein was processed in mitochondria of animals from day 4 and significantly increased mitochondrial functionality in the hearts of 21-days treated animals. Additional studies are currently performed in the well-characterized conditional mouse model of complete Frataxin deletion in cardiac and skeletal muscles (Mck-Cre-FxnL3/L– mice, aka "Puccio" model) which demonstrates most features of FA cardiomyopathy.

In conclusion, BB-FA (TAT-MTS(cs)-Frataxin), exhibits promising potential as a protein replacement drug candidate for FA. Our findings offer a new platform for protein replacement in the treatment of various genetic mitochondrial metabolic disorders characterized by deficiency of a functional critical protein, diseases which currently have no cure.

## The neuroprotective and neuroregenerative properties of bone marrow stem cell mobilising drugs in Friedreich's ataxia

Kevin Kemp, Neil Scolding, Alastair Wilkins

*University of Bristol, United Kingdom*

Despite the large amount of research into pathogenic mechanisms which operate in Friedreich's ataxia, at the present time, therapies show little ability to protect nerves specifically and no capacity to promote neuroregeneration. A large body of experimental evidence, including our own, has indicated that bone marrow-derived stem and progenitor cell populations show therapeutic promise. They represent a cell therapy that is likely to have a real impact in neurological diseases and they act via multiple mechanisms which are particularly apposite to a disease such as Friedreich's ataxia.

We have performed a series of experiments showing that the bone marrow stem cell mobilising drugs (granulocyte-colony stimulating factor (G-CSF) and stem cell factor (SCF)), display strong neuroprotective properties and activate cell survival pathways in mature neurons and in cells derived from patients with Friedreich's ataxia. Furthermore, the administration of both G-CSF and SCF in the YG8R mouse model of Friedreich's ataxia leads to improvement in the neurological phenotype associated with the disease. Mice treated over a six month period show significant improvements in motor phenotype using an accelerating rotarod, grip strength, string test and open field, when compared to untreated controls. In addition, using bone marrow transplantation in the YG8R mouse model, to establish bone marrow chimeric mice that stably express both enhance green fluroescent protein (EGFP) and 'normal' copies of the frataxin gene, we also show that GCSF/SCF treatment stimulates bone marrow-derived cells carrying the 'normal' frataxin gene to enter the peripheral circulation and subsequently migrate throughout the central nervous system (including the dorsal root ganglia, cerebellum and spinal cord) where they co-express either neuronal or glial cell markers, providing a link to functional motor recovery.

In summary, our studies have provided novel and fundamental insights into the ways in which nerve cells in Friedreich's ataxia can be protected or replaced and their survival prolonged with the administration of stem cell mobilising drugs. We have also shown that the induced migration of bone marrow-derived cells into the CNS could indeed serve as a tool to aid the delivery 'healthy donor' cells and/or frataxin genes to sites of CNS injury. We therefore propose that administration of stem cell mobilising drugs may have the potential to be developed into a simple, non-invasive and effective neuroprotective and regenerative therapy in patients with Friedreich's ataxia.

## Stabilization of FXN mRNA Using Oligonucleotides for the Treatment of Friedreich's ataxia

Fatih Ozsolak, Kamaljeet Sandhu, Susan Wood, David Bullough, Jim Barsoum, Paula Lewis

*RaNA Therapeutics, United States*

Friedreich's ataxia (FRDA) is a recessively inherited neuromuscular disorder that arises due to cellular depletion of frataxin (FXN) protein and resulting defects in mitochondrial functions. The protein coding sequence of FXN is normal in the majority of FRDA patients, suggesting that upregulation of endogenous FXN expression could be an effective therapy. The most common molecular cause of this disease is the expansion of GAA/TTC triplet repeats in the first intron of FXN gene. Repeat expansion beyond a certain threshold causes defects which reduce FXN mRNA and protein levels. DNA-DNA and DNA-RNA interactions formed in the long triplet repeat stretches, defects and alterations in splicing patterns and the formation of a heterochromatin-like structure are among the potential causes of repeat-induced FXN silencing. We developed a novel oligotherapeutics-based strategy to upregulate genes by targeting mRNA end regions. This strategy likely involves acting at the post-transcriptional level and is therefore independent of GAA-repeat induced mechanistic changes and defects. We applied this strategy to FXN mRNA and observed significant upregulation in both FRDA cells in vitro and in a FRDA mouse model. This oligonucleotide-based therapeutic approach represents a novel strategy for the treatment of FRDA and other human diseases.

## Genetic and pharmacological rescues of spinocerebellar ataxia in the SCA28 model open to human therapy

Francesca Maltecca[1, 2], Elisa Baseggio[1, 2], Francesco Consolato[1, 2], Davide Mazza[2], Paola Podini[2], Samuel M. Young[3], Ilaria Drago[4, 5], Ben A. Bahr[6], Aldamaria Puliti[7], Franca Codazzi[1, 2], Angelo Quattrini[2], Giorgio Casari[1, 2]

*[1] Università Vita-Salute San Raffaele, Italy, [2] San Raffaele Scientific Institute, Italy, [3] Max Planck Florida Institute for Neuroscience, US, [4] University of Padua, Italy, [5] The Scripps Research Institute Florida, US, [6] University of North Carolina-Pembroke, US, [7] University of Genoa, Italy*

Spinocerebellar ataxia type 28 (SCA28) is a neurodegenerative disorder characterized by unbalanced standing, gait incoordination, nystagmus, ophthalmoparesis and pyramidal signs. Several disease-causing mutations have been identified in the AFG3L2 gene. The encoded protein, AFG3L2, coassembles with paraplegin into multimeric complexes, called *m*-AAA proteases, in the inner mitochondrial membrane. These complexes are crucial components of the mitochondrial protein quality control system and regulate mitochondrial morphology.

The haploinsufficient *Afg3l2*$^{+/-}$ mouse recapitulates the features of SCA28 patients, displaying motor incoordination due to dark degeneration of Purkinje cells (PC-DCD). This is a form of degeneration shared by several genetic forms of SCA and is characterized by toxic levels of intracellular calcium and activation of calpains. Peculiarly, in the SCA28 mouse this phenomenon is unique since it originates from mitochondrial dysfunction.

We estabilished in cultured PCs that *Afg3l2*-depleted mitochondria ineffectively buffer the evoked calcium peaks, thus enhancing cytoplasmic calcium levels and finally triggering PC-DCD. This defect is caused by the negative synergism between mitochondrial depolarization and altered trafficking of the organelles to PC dendrites.

Supporting this mechanism, we completely recover the ataxic phenotype of SCA28 mice by genetically reducing the metabotropic glutamate receptors mGluR1, and thus decreased calcium influx in PCs. The same result has been successfully replicated by a pharmacological treatment with the cefalosporin ceftriaxone, which was shown to increase consistently the transcription levels of the astrocyte glutamate transporter EAAT2. On the basis of the SCA28 pathogenetic mechanism that we defined, we used this drug to reduce the glutamatergic stimulation of PCs in *Afg3l2*$^{+/-}$ mice. This treatment is effective when applied at both presymptomatic and after the ataxia onset in the preclinical model, thus representing a safe and immediately accessible therapy for pre-symptomatic carriers of AFG3L2 mutations and also SCA28 patients with overt symptoms. Moreover, ceftriaxone could represent a therapeutic perspective for the subset of SCA characterized by PC-DCD and dysregulation of cerebellar calcium homeostasis.

## Transplantation of cerebellar neural stem cells alleviates motor coordination and neuropathological deficits of a transgenic mouse model of Machado-Joseph disease

Liliana Mendonça[1], Clévio Nobrega[1], Hirokazu Hirai[2], Brian Kaspar[3], Luis Pereira de Almeida[1, 4]

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Machado-Joseph disease or Spinocerebellar Ataxia type 3 is a neurodegenerative disease for which no diasease-modifying treatment is available. Machado-Joseph disease patients exhibit significant motor impairments such as gait ataxia, associated to multiple neuropathological changes including mutant ataxin-3 inclusions, marked neuronal loss and atrophy of the cerebellum. Therefore, an effective treatment of symptomatic Machado-Joseph disease patients may require cell replacement, which we investigated in this work.

For this purpose, we injected cerebellar neural stem cells (cNSC) in the cerebellum of adult Machado-Joseph disease transgenic mice and assessed the effects in neuropathology, neuroinflammation mediators and neurotrophic factor levels and motor coordination. We found that upon transplantation into the cerebellum of adult Machado-Joseph disease mice, cNSC differentiate into neurons, astrocytes and oligodendrocytes. Importantly, cNSC transplantation mediated a significant and robust alleviation of the motor behavior impairments, which correlated with a preservation from Machado-Joseph disease associated neuropathology, namely reduction of Purkinje cells loss, reduction of cellular layers shrinkage and mutant ataxin-3 aggregates.

Additionally, a significant reduction of neuroinflammation and an increase of neurotrophic factors levels were observed, indicating that transplantation of cNSC also triggers important neuroprotective effects. Overall, these data suggest that cNSC have the potential to be used as a cell replacement and neuroprotective approach for Machado-Joseph disease therapy.

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## Therapeutic strategies to prevent the ubiquitin/proteasome-dependent degradation of frataxin

Alessandra Rufini[1, 2], Silvia Fortuni[1, 2], Monica Benini[1], Francesca Cavallo[1], Ivano Condò[1], Gabriella De Martino[1], Ottaviano Incani[1], Damiano Sergio Massaro[1], Giulia Alfedi[1], Giorgia Alaimo[1], Almerinda Di Venere[3], Florence Malisan[1], Dario Serio[1], Roberto Testi[1, 2]

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Frataxin levels critically affect onset and progression of Friedreich ataxia. Our therapeutic approaches are therefore aimed at increasing frataxin levels. This can be in principle achieved by increasing the transcription rate or by interfering with its degradation. We previously discovered that a significant amount of frataxin is degraded by the ubiquitin/proteasome system before it reaches mitochondria and we identified the critical ubiquitination site on frataxin. We then described the therapeutic potential of small molecules that increase frataxin levels by docking on the frataxin ubiquitination site, thus preventing frataxin ubiquitination and degradation. We called these compounds ubiquitin-competing molecules (UCM). Through an iterative process of computational docking, chemical synthesis and cell-based functional assays, we identified a set of compounds that efficiently promote frataxin accumulation. These compounds directly interact with frataxin and prevent its ubiquitination. Most importantly, these compounds are able to promote frataxin accumulation and aconitase rescue in patients-derived cells, strongly suggesting their therapeutic potential.

In light of these results, another attractive therapeutic strategy to increase frataxin levels would be the inhibition of the enzyme responsible for its ubiquitination. To identify the frataxin-specific E3 ligase we performed a siRNA-based functional screening of an E3 ligase-restricted siRNA library, targeting more than 600 different genes. Knock-down of the frataxin-specific E3 ligase is expected to result in the accumulation of frataxin protein. Through this screening procedure we isolated one gene that consistently promotes frataxin accumulation when its expression is silenced in cells. Importantly silencing of this gene induces frataxin accumulation also in fibroblasts derived from patients. Moreover, the overexpression of the corresponding cDNA, but not its catalytic inactive mutant, promotes frataxin ubiquitination. This gene may actually code for the enzyme responsible for frataxin ubiquitination and may represent a novel therapeutic target for Friedreich ataxia.

Together our data indicate that the strategy aimed at preventing the ubiquitin/proteasome-dependent degradation of frataxin has therapeutic potential for FRDA.

## An AAV9 coding for frataxin clearly improved the symptoms and prolonged the life of Friedreich ataxia mouse models

Catherine Gérard[1], Xiao Xiao[2], Mohammed Filali[1], Pierre Chapdelaine[1], Marie Arsenault[3], Jacques P. Tremblay[1]

*[1] Centre de Recherche du Centre Hospitalier Universitaire de Québec and Department of Molecular Medecine, Faculty of Medecine, Laval University, Canada, [2] Division of Molecular Pharmaceutics, UNC Eshelman School of Pharmacy, US, [3] Centre de recherche, Institut universitaire de cardiologie et de pneumologie de Québec, Canada*

Friedreich ataxia (FRDA) is a genetic disease due to increased repeats of the GAA trinucleotide in intron 1 of the frataxin gene. This mutation leads to a reduced expression of frataxin. We have produced an AAV9 coding for human frataxin (AAV9-hFXN). This AAV was delivered by intra-peritoneal injection to young conditionally knockout mice in which the frataxin gene had been knocked-out in some tissues during embryogenesis by breeding them with mice expressing the Cre recombinase gene under the MCK or the NSE promoter. In the first part of the study different doses of virus (i.e., 6x1011 v.p. to 6x109 v.p.) were tested from in NSE-cre mice. All doses led to an increase in life spenttime of the mice. The higher and the lower dose were also tested in MCK-cre mice. A single administration of the AAV9-hFXN at 6x1011 v.p. more than doubled the life of these MCK-cre mice. In fact the MCK-cre mice treated with the AAV9-hFXN were sacrificed for further molecular investigations at the age of 29 weeks without apparent symptoms. Echography analysis of the heart function clearly indicated that the cardiac systolic function was better preserved in the mice that received 6x1011 v.p. of AAV9-hFXN. The human frataxin protein was detected by ELISA in the heart, brain, muscles, kidney and liver with the higher dose of virus in both mouseice models. Thus gene therapy with an AAV9-hFXN is a potential treatment of FRDA.

*This work was supported by grants from Ataxie Canada and from Association Française de l'Ataxie de Friedreich.*

## Therapeutic role of Neuropeptide Y in mouse models of Machado-Joseph Disease

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Machado-Joseph disease (MJD) is a dominantly-inherited neurodegenerative disorder associated with an expanded polyglutamine tract within the protein ataxin-3. This mutated protein causes progressive impairment of motor coordination, associated to the neurodegeneration of cerebellum, pons, brain stem, substantia nigra and striatum. The currently available therapies do not allow modification of disease progression.

Neuropeptide Y (NPY) and NPY receptors are widely distributed in the CNS. Moreover, NPY has been shown to exert neuroprotective effects. Thus, in the present work we investigated the impact of NPY on neurochemical and behavioural modifications in rodent models of MJD.

We analysed whether NPY levels are altered in post mortem patient brain tissue and animal models of MJD. Additionally, we evaluated the impact of NPY overexpression in the striatum and cerebellum of a lentiviral-based model and a transgenic model of MJD, respectively, through stereotaxic injection of adeno-associated viral vectors (AAVs) encoding either NPY, or EGFP as control. Lentiviral-based MJD animals were sacrificed at 4 and 8 weeks post-surgeries for immunohistochemical and western blot analysis of mutant ataxin-3 inclusions and DARPP-32 staining. Motor behaviour defects of MJD transgenic animals were evaluated by stationary rotarod, beam walking and footprint patterns, before and 4 and 8 weeks after AAV-injections.

NPY mRNA levels of brain extracts of both post mortem patient brain tissue (dentate nucleus, n=2) and mice (striatum of lentiviral-mediated overexpressing mutant-atx3 animals, and dissected cerebella of transgenic MJD) were significantly decreased. Additionally, we observed a significant decrease in the number of NPY-positive interneurons upon striatal overexpression of mutant atx3 in the lentiviral-based MJD model, justifying the reinstatement of NPY levels.

NPY overexpression in the striatum of the MJD lentiviral model mediated a significant decrease in the number of mutant ataxin-3 inclusions and a 55 ± 9% reduction of the striatal lesion assessed by DARPP-32 immunoreactivity. In addition, NPY overexpression in cerebella of transgenic MJD mice reduced motor behaviour defects, resulting in: i) reduction in the latency to fall of the rod; ii) improvement to cross the round beams; and iii) almost complete rescue of footprint overlap.

Taken together, these data show that NPY is reduced in MJD and that NPY overexpression reduces MJD-associated neuropathology and motor-related deficits, which supportsNPY overexpression as a candidate strategy to modulate and prevent the abnormal neurochemical and motor changes in MJD.

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## Src inhibitors modulate frataxin protein levels

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Defective expression of frataxin is responsible for the inherited, progressive degenerative disease Friedreich's Ataxia (FRDA). There is currently no effective approved treatment for FRDA and patients die prematurely. Defective frataxin expression causes critical metabolic changes, including redox imbalance and ATP deficiency. Since these alterations are known to activate the tyrosine kinase Src, we investigated whether Src might in turn affect frataxin expression. We found that frataxin can be phosphorylated by Src. Phosphorylation occurs primarily on Y118 and promotes frataxin ubiquitination, a signal for degradation. Accordingly, Src inhibitors induce accumulation of frataxin but are ineffective on a non-phosphorylatable frataxin-Y118F mutant. Importantly, all the Src inhibitors tested, some of them already in the clinic, increase frataxin expression in frataxin-deficient cells derived from FRDA patients. Thus, Src inhibitors emerge as a new class of drugs able to promote frataxin accumulation, suggesting their possible use as therapeutics in FRDA.

# Session 6: Biomarkers and functional measures

## Saturday 28th March

*Chairs: David Lynch (University of Pennsylvania, US) and Bernard Ravina (Voyager Therapeutics, USA)*

Invited speaker

8:40 David Lynch (University of Pennsylvania, USA):
*Biomarkers in Friedreich ataxia*

Selected presentations

9:10 Ian Blair (University of Pennsylvania, Philadelphia, US):
*Platelet biomarkers of metabolic disturbances in Friedreich's ataxia*

9:25 Martin Delatycki (Murdoch Childrens Research Institute, Parkville, Australia):
*A longitudinal study of the Friedreich ataxia impact scale*

9:40 Zofia Fleszar (Hertie-Institute for Clinical Brain Research, Tübingen, Germany):
*PreAtaxia: characterizing ataxia-specific movement changes at a preclinical stage*

9:55 Louise Corben (Monash University, Melbourne, Australia):
*Abnormal brain function and connectivity in cerebello-cerebral circuits underlying cognitive function in Friedreich ataxia: The IMAGE-FRDA study*

10:10 Pierre-Gilles Henry (University of Minnesota, US):
*MRS and diffusion MRI of the spinal cord in Friedreich's ataxia*

10:25 Break

10:55 Marinela Vavla (E. Medea Scientific Research Institute, Conegliano and Bosisio Parini, Italy):
*Potential neuroimaging biomarkers validated in Friedreich's ataxia: DTI and functional magnetic resonance findings*

11:10 Mafalda Raposo (Centre of Research in Natural Resources, University of the Azores, Portugal):
*Novel candidate blood-based transcription biomarkers of spinocerebellar ataxia type 3 (SCA3)*

11:25 Louise Corben (Physiotherapy Department, Monash Health, Cheltenham, Australia):
*Sensitivity of spatiotemporal gait parameters in Friedreich ataxia*

11:40 Michael Parkinson (UCL Institute of Neurology, UK):
*OCT in Diagnosing ARSACS*

11:55 R. Mark Payne (Indiana University School of Medicine, Indianapolis, US):
*Fatty acid oxidation is disrupted in the FRDA heart*

12:15 Jorg B. Schulz (Department of Neurology, RWTH Aachen University, Germany):
*Biological and clinical characteristics of the European Friedreich Ataxia Consortium for Translational Studies (EFACTS): cross-sectional analysis of baseline data*

12:30 Martin Delatycki (Murdoch Childrens Research Institute, Melbourne, Australia):
*The views of individuals with, and parents of individuals with Friedreich ataxia regarding pre-symptomatic testing of minors*

12:45 Close of session

Invited Speaker: David Lynch (University of Pennsylvania, USA)

## Biomarkers in Friedreich ataxia

Friedreich ataxia (FRDA) is an autosomal recessive ataxia with early onset reflecting the deficiency of functional frataxin in cells. As much is understood about the mechanisms of disease in FRDA, many agents are in therapeutic development. This process would be aided by development of biomarkers of disease progression. This presentation will review a series of anatomical and functional biomarkers of frataxin deficiency.

Physiological and anatomical biomarkers are crucial for assessment in FRDA, in particular defining the state of neurological abilities and the structural components of cell loss. Imaging studies have previously used MRI of brain and spinal cord to identify cell loss and other anatomic changes. Modalities like optical coherence tomography can be used to investigate the retina selectively and detailed physiology (SSEP, BAER, LiSN-S etc.) maybe useful for identifying abnormal neural properties earlier than clinical evaluation. However these markers do not reveal changes in the pathophysiological process at their earliest point. Identifying biomarkers of pathophysiology is necessary for assessing the effect of molecular therapy.

At the most basic pathophysiological level, cellular frataxin levels provide a unifying biomarker in FRDA. Frataxin deficiency can be measured in a variety of peripheral tissues, and levels correlate with the GAA repeat length on the shorter allele. Most point mutations in FRDA also give rise to lower levels of frataxin protein. Frataxin levels appear to be constant over the course of the disease. However, the degree to which levels in unaffected tissue reflect levels in affected tissues is unclear. In addition, assessment of frataxin is useful mainly for therapies designed to raise frataxin levels, and is without benefit in assessment of downstream pathways.

A second approach is to examine metabolic dysfunction created by frataxin deficiency. Such approaches could concentrate on levels of Krebs cycle intermediates, or other key metabolic species. Using mass spectrometry based approaches; these measurements have the potential to be extraordinarily sensitive. They would be even more useful if they could be combined with anatomical resolution to create novel imaging modalities. Collectively, these finding illustrate the need for a diverse effort in biomarker development in FRDA while being sensitive to the need for efficient use of resources to identify the most useful ones.

## Platelet biomarkers of metabolic disturbances in Friedreich's ataxia

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Friedreich's ataxia (FRDA) is a heritable disease characterized by spinocerebellar degeneration and cardiomyopathy, with metabolic abnormalities that are suspected to have a role in disease pathogenesis. Despite this knowledge, the inability to access the highly affected neuronal and cardiac tissues has hampered metabolic evaluation and biomarker development. In this study, we used platelets from patients with FRDA coupled with liquid-chromatography-mass spectrometry methodology to assess their ability to metabolize stable isotope-labeled glucose and palmitate to acyl-coenzyme A (CoA) isotpologues associated with mitochondrial metabolism. Our findings revealed that platelets from FRDA patients (n=10) had diminished relative incorporation of [$^{13}C_6$]-glucose into the Krebs cycle through acetyl-CoA when compared with control subjects (n=10). In addition, the decrease of labeling into acetyl-CoA showed a negative correlation with GAA repeat length ($r^2$ = 0.39), a known marker of disease severity. This is consistent with studies that have shown diminished pyruvate oxidation in FRDA. In addition to decreased glycolysis, we observed a concomitant increase in the β-oxidation of fatty acids. This was revealed by a shift in metabolism by FRDA platelets toward formation of β-hydroxybutyryl (βHB)-CoA and 3-hydroxy-3-methyl-glutaryl (HMG)-CoA from [$^{13}C_{16}$]-palmitate in FRDA platelets when compared with controls. In contrast to the [$^{13}C_6$]-glucose-derived acetyl-CoA, there was a positive correlation of [$^{13}C_{16}$]-palmitate labeling into βHB-CoA with GAA repeat length ($r^2$ = 0.51). The β-oxidation of fatty acids results in the removal of contiguous two carbon units from the fatty acyl substrate to generate acetyl-CoA, which can then enter into the Krebs cycle. Consequently, alterations to lipid metabolism might play an important role in cellular homeostasis in times of mitochondrial dysfunction. Taken together these results suggest FRDA platelets exhibit a diminished capacity for oxidative phosphorylation, as decreased [$^{13}C_6$]-glucose labeling into acetyl-CoA has been shown to occur in response to pharmacologic inhibition of mitochondrial complex I. Furthermore, our previous cell culture studies have shown increased β-oxidation of lipids in response to diminished complex I activity, supporting the notion that lipid breakdown plays an important compensatory role in times of mitochondrial dysfunction. Finally, generation of a receiver operator characteristic (ROC) curve combining decreased labeling into acetyl-CoA from [$^{13}C_6$]-glucose together with increased labeling into βHB-CoA from [$^{13}C_{16}$]-palmitate revealed an area under the curve of 0.90. Therefore, our findings demonstrate that platelets can be used as a surrogate tissue for in vivo metabolic studies and lend insight into metabolic defects in heritable mitochondrial and metabolic diseases such as FRDA.

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## A longitudinal study of the Friedreich ataxia impact scale

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**Background:** Quality of life in Friedreich ataxia (FRDA) has been explored using various generic health status measurement tools, most commonly the Short Form Health Survey Version 2 (SF36v2). The tool did not address many specific issues related to disease impact in people with FRDA. The Friedreich Ataxia Impact Scale (FAIS) was developed to examine clinically relevant areas in FRDA. The aims of the current study were to assess the relationship between the FAIS and clinical characteristics of FRDA, as well as to determine the responsiveness of the FAIS to change over one and two years.

**Methods:** One hundred and four individuals with FRDA aged at least 18 years and homozygous for the GAA expansion in intron 1 of FXN, completed the FAIS at baseline. Seventy individuals completed the FAIS again 12 months later and 49 completed the FAIS at 24 months. Clinical parameters and neurologic scales (Friedreich Ataxia Rating Scale (FARS)) were also recorded.

The FAIS comprises 126 items grouped into eight independent subscales, measuring three areas identified as being clinically important to individuals with FRDA: 1) symptoms, 2) physical functioning, and 3) psychological and social impact. Symptoms encompass speech and body movement. The FAIS was designed to be used together with current clinician-administered rating scales to capture the true health impact of FRDA.

Spearman's rank correlation coefficients were utilised to correlate the FAIS subscales with disease parameters; these included age at disease onset, disease duration, GAA1 and GAA2 repeat sizes Friedreich Ataxia Rating Scale (FARS) score. The FAIS subscales were also correlated with the Physical Component Summary (PCS) and the Mental Component Summary (MCS) of Version 2 of the SF-36. Responsiveness was examined by measuring the change in median subscale scores using Wilcoxon signed-rank test between baseline and 12 months, and between baseline and 24 months.

**Results:** The total FARS score, onset age and disease duration correlated significantly with FAIS subscales measuring symptoms and physical functioning. There were no significant correlations between GAA1 or GAA2 repeat sizes and any of the FAIS subscales. Both summary measures of the SF-36V2 also correlated well with the FAIS subscales. Speech was the only subscale that demonstrated significant change over one and two years.

**Conclusions:** The FAIS provides valuable insight into the perspective of individuals with FRDA on their health status, and is an important measure of morbidity. It has, however, limited responsiveness to change and its use in intervention studies is questionable.

## *PreAtaxia*: Characterizing ataxia-specific movement changes at a preclinical stage

Zofia Fleszar[1, 2, 3, 4], Cornelia Schatton[1, 2], Martin Giese[1, 2], Ludger Schöls[3, 4], Winfried Ilg[1, 2], Matthis Synofzik[3, 4]

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**Background**: It is well-known from many neurodegenerative movement disorders, that at the point of clinical manifestation, large populations of underlying neurons are already lost and most compensatory resources exhausted. Thus, effectiveness of future interventions and their evaluation will largely depend on (i) detecting these diseases as early as possible, (ii) a more detailed understanding of the development of dysfunctional motor control mechanisms and compensation strategies within the pre-symptomatic stage. Here, we aimed to establish measures that allow the identification of ataxia-specific dysfunctions already in the earliest stage of degenerative ataxia. Specifically, we hypothesized that an increasing complexity of balance and gait tasks might reveal dysfunctions in pre-clinical stages of the disease.

**Methods**: We assessed (i) stance (Romberg test) in different complexities including closed eyes and on an elastic mat and (ii) walking on hard and soft ground. Ataxia-specific movement changes including spatial and temporal variability[1] as well as body sway were assessed by quantitative movement analysis. Assessments were performed in three groups: 1.) Group EARLY: N=21 patients with early stage degenerative ataxia [SARA score: 3-7 points]; 2.) Group PRE: N=11 subjects with premanifest ataxia [SARA score <3 points, 8 of 11 mutation carriers for spinocerebellar ataxia (SCA) types 1,2,3 or 6]; 3.) Group CONTROL: N=25 age-matched healthy controls. For SCA mutation carriers in the PRE group, movement parameters were related to genetically estimated clinical disease onset[2].

**Results**: A significant difference in body sway was observed in all Romberg conditions between the groups EARLY and HC ($p<0.001$) as well as between EARLY and PRE ($p<0.02$). Differences between PRE and CONTROL have been identified in classical Romberg, with closed eyes, and on the mat with closed eyes ($p<0.002$). In gait, we found no single measure differentiating groups PRE and CONTROL. This might be due to differences in gait strategies (e.g. velocity, step width), which overwhelm subtle changes in the PRE group. By using multi-variate features analysis (logistic regression models), we succeeded in identifying feature sets that capture these strategies and enable a significant differentiation between PRE and CONTROL subjects ($p=0.02$). Furthermore, a correlation ($p=0.03$) between model outputs of movement parameters for SCA subjects and their estimated disease onset[2] indicates a continuous development in the pre-symptomatic phase of the disease.

**Discussion**: We were able to identify movement features in stance and gait, which differentiate healthy controls not only from early ataxia subjects, but also from pre-clinical ataxia subjects. Furthermore, these features show a correlation for stance and gait changes to the time of estimated disease onset, thus describing a continuum of change already within the pre-clinical phase. These findings will establish the basis for therapeutic trials aiming to delay disease onset and progression in premanifest gene carriers of SCA.

*References*

1. Ilg W et al. Brain 2007;130(3):786-798

2. Tezenas du Montcel S et al. Journal of medical genetics 2014

## Abnormal brain function and connectivity in cerebello-cerebral circuits underlying cognitive function in Friedreich ataxia: The IMAGE-FRDA study

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**Introduction:** Within the brain, the principal consequence of Friedreich ataxia (FRDA) involves the progressive degeneration of the dentate nucleus of the cerebellum. Beyond classically reported motor and sensory symptoms resulting from dentate atrophy, there is increasing acknowledgement that some degree of cognitive impairment also defines the gross phenomenology of the condition. In particular, FRDA has been associated with deficits in working memory, attention, and cognitive control, processes that rely on intact interactions between the cerebellum and prefrontal cerebral cortices. Disruption to cerebello-thalamo-cerebral connectivity may therefore underlie changes to cognitive functioning in FRDA. This study examined the integrity of brain activity and connectivity within cerebello-thalamo-cerebral systems in individuals with FRDA while undergoing a working memory task.

**Methods:** Twenty-nine individuals homozygous for a GAA expansion in intron 1 of FXN, and 34 matched control participants undertook a functional magnetic resonance imaging (fMRI) protocol. During scanning, participants performed an N-Back working memory task with two levels of cognitive load. Task stimuli consisted of a sequential string of letters, each presented visually for 500ms and separated by 1500ms. The low-load ("0-Back") condition required participants to indicate, via button press, when a pre-instructed letter appeared on screen. The high-load ("2-Back") condition necessitated a button press when the current letter was the same as that presented two letters previously. To isolate brain activations related to higher-order cognitive processing, group differences in the magnitude of the fMRI signal during 2-Back performance was contrasted with the 0-Back condition using gold-standard statistical parametric mapping (SPM) approaches. Cerebello-cerebral functional interactions were inferred based on the covariation of task-related fMRI signals in the cerebellum and the cerebrum.

**Results:** Behaviourally, there were no significant group differences in reaction time or error rates when 2-Back was contrasted with 0-Back. The imaging data also showed qualitatively similar areas of functional activation across both groups; however, individuals with FRDA showed significantly reduced brain activations in cognitive regions of the cerebellar cortex (i.e., Lobule VI) and associated cerebral cortices, including the anterior insula and lateral prefrontal cortex. Moreover, in individuals with FRDA, the functional connectivity between these regions was significantly reduced, and normal patterns of task-related connectivity dynamics were diminished, as compared to controls. All results are statistically significant at family-wise error corrected $p < 0.05$.

**Conclusions:** These results provide evidence that cerebellar pathology in FRDA directly links with changes in cerebral activation and connectivity during working memory performance. Taken together, this study supports the conceptualization of FRDA as a disorder of large-scale, spatially distributed cerebral and cerebellar circuitry, providing further explanation for the non-motor symptoms associated with this disease.

## MRS and diffusion MRI of the spinal cord in Friedreich's ataxia

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**Purpose**: Although spinal cord atrophy is a hallmark of Friedreich's ataxia (FRDA), there have been very few MR studies of the spinal cord in patients with FRDA[1] and, to our knowledge, none using $^{1}$H MRS or DTI, due in part to technical challenges ($B_0$ shim, motion artifacts). Here, our objective was to characterize neurodegeneration in early stage patients with FRDA using $^{1}$H MRS and DTI of the spinal cord.

**Methods**: We studied 15 patients and 15 age-matched controls. All measurements were performed on a Siemens Trio 3T scanner (Siemens, Erlangen, Germany). $^{1}$H MR spectra (TE = 28 ms, TR = 5 s, 256 averages) were acquired using a modified semi-LASER sequence[2] in an 8 × 6 × 30 mm$^3$ voxel positioned along C4-C5 vertebrae. Spectra were quantified with LCModel using water as an internal reference. Diffusion MRI at the C2-C3 level was acquired using a readout-segmented echo-planar sequence[3] with 1.1x1.1x3.3mm$^3$ resolution and with correction of geometric and eddy current distortions[4]. All subjects were also assessed by the Friedreich's Ataxia Rating Scale (FARS).

**Results**: Patients had FARS scores averaging 45±17 (mean ± SD, range 10-81) and age 20±7 years (range 11-32). We observed 33% lower NAA ($p<1e-5$) and 32% higher myo-inositol ($p<0.005$) levels in spinal cord of patients vs controls, reflecting neuronal damage and gliosis. Similarly, fractional anisotropy was lower in the cervical spinal cord of patients (FA = 0.46±0.04 in patients vs. 0.59±0.06 in controls, $p < 0.001$), reflecting alteration of axonal integrity. In spite of the large differences observed between controls and patients, there was no correlation between these parameters and FARS scores on this small group of patients, suggesting that these changes occur very early in the disease process, possibly even before the apparition of clinical symptoms. The number of fibers, however, correlated negatively with FARS, likely reflecting spinal atrophy.

**New results to be presented at the ARC 2015 meeting**: The second year of this MR study is focused on a) 12-month follow-up of patients scanned in 2013-2014; b) recruitment of additional early stage patients; and 3) assessment of the precision of MR parameters (test-retest). These data are currently being acquired (as of Nov 2014). Therefore the results are not available for this abstract but will be available for presentation at the meeting.

**Conclusion**: This is, to our knowledge, the first report using 1H MRS or DTI to study spinal cord in patients with FRDA. Such multi-modal MRI/S measurements in the spinal cord may yield further insight into disease mechanisms and provide markers of neurodegeneration in patients at an early stage to assess therapeutic efficacy in clinical trials.

*References:*
[1] Chevis Cereb 2013 [2] Oz Magn Reson Med 2011 [3] Porter MRM 2009 [4] Andersson Proc ISMRM 2012

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## Potential neuroimaging biomarkers validated in Friedreich's ataxia: DTI and functional magnetic resonance findings

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**Background**: Friedreich's ataxia (FRDA) is a progressive hereditary neurodegenerative condition caused by an autosomal recessively inherited GAA repeat in the FXN gene. In this study we used clinical measures and advanced tractography combined to functional MRI (fMRI) to explore white matter (WM) connectivity and motor dysfunction in a cohort of FRDA patients.

**Methods**: Molecularly defined FRDA patients (n=17) were clinically assessed with the specific ataxia scales. Patients and age matched healthy controls underwent a neuroimaging study protocol on a 3T MRI scanner that included advanced neuroimaging DTI and fMRI. After the pre-processing, a nonlinear monoexponential model was used to calculate fractional anisotropy (FA), mean, radial and axial diffusivity (MD, RD, AD) maps. Non-parametric voxel-based permutations were performed on the WM maps regions of interest (ROI), considering age and sex via a general linear model (GLM) with critical threshold 0.05 while correcting for multiple tests. An fMRI sequence was acquired during a simple block design finger-tapping task. After a standard pipeline pre-process, intra- and intergroup GLM analysis were conducted, considering age and sex variables and also $p < 0.001$ threshold.

**Results**: Our cohort included early onset FRDA patients, mean age at onset 10.65 ± 5.08 (range 4-20 years); F/M: 13/4; mean GAA expansion in the smaller repeat was 651,07 ± 234.39 (n=16) and one patients with a single base pair deletion and 170 GAA repeat. Mean age at assessment was 27.82 ± 10.51years (12-51), mean disease duration was 17.17 ± 8.43 (4-33). The mean age of the control group was 23 ± 4.83 years; F/M= 5/8. From both the voxel-based and ROI-based analysis altered FA and MD parameters were consistently found in the following four Central Nervous System areas: cerebellar WM (superior, median and inferior peduncles), long sensory-motor pathways (corticospinal and lemnisceal systems, cerebral peduncles), major commissural fibres (splenium and tapetum of the corpus callosum), the thalamic and the optic radiations. The fMRI data were analyzed from 13 patients (mean age 30.05 ± 11.76 years) and 8 controls (mean age 24.5 ± 3.85 years). The finger-tapping task demonstrated intragroup activation of the controlateral motor cortex and the ipsilateral cerebellar cortex both in patients and healthy controls. Intergroup analysis demonstrated a consistent and significantly higher cerebellar cortex activation, in controls compared to the FRDA patients, in particular in the lobules V and VI.

**Discussion**: We show that a comprehensive MRI protocol consistently discriminates FRDA patients from controls. DTI changes in selected areas and BOLD signal in the cerebellar ipsilateral cerebellar cortex in response to a simple motor task show strong intergroup discriminating power and may prove to be useful paraclinical disease markers. A longitudinal study is undergoing to explore the sensitivity of these indicators to disease progression.

## Novel candidate blood-based transcriptional biomarkers of spinocerebellar ataxia type 3 (SCA3)

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Spinocerebellar ataxia type 3 (SCA3; or Machado-Joseph disease, MJD) is a late-onset polyglutamine neurodegenerative disorder, caused by a mutation in the *ATXN3* gene, which encodes for the ubiquitously expressed protein ataxin-3. Previous studies on cell and animal models have suggested that mutated ataxin-3 is involved in transcriptional dysregulation. Starting with a whole-transcriptome profiling of peripheral blood samples from patients and controls, we aimed to confirm abnormal expression profiles in SCA3 and to identify promising up-regulated genes as potential candidate biomarkers of disease status. The Illumina Human V4-HT12 array was used to measure transcriptome-wide gene expression in peripheral blood samples from 12 patients and 12 controls. Technical validation and validation in an independent set of samples were performed by quantitative real-time PCR. Based on the results from the microarray, twenty six genes, found to be up-regulated in patients, were selected for technical validation by quantitative real-time PCR (validation rate of 81% for the up-regulation trend). Fourteen of these were further tested in an independent set of 42 patients and 35 controls; ten genes maintained the up-regulation trend (*FCGR3B*, *CSR2RA*, *CLC*, *TNFSF14, SLA, P2RY13, FPR2, SELPLG, YIPF6* and *GPR96*); *FCGR3B, P2RY13 and SELPLG* were significantly up-regulated in patients when compared to controls. Our findings support the hypothesis that mutated ataxin-3 is associated with transcription dysregulation, detectable in peripheral blood cells. Furthermore, this is the first report suggesting a pool of up-regulated genes in SCA3, which may have the potential to be used for fine phenotyping of this disease.

## Sensitivity of spatiotemporal gait parameters in Friedreich ataxia

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Friedreich ataxia (FRDA) is an autosomal recessive disease with average symptom onset between 10-15 years of age. Initial symptoms are 'clumsiness' and gait ataxia, however mobility progressively declines and people with FRDA typically become non-ambulant 10 to 15 years after disease onset. Loss of ambulation has a significant impact on quality of life in people with FRDA. Thus a more comprehensive understanding of gait dysfunction will provide a better basis for targeting specific therapeutic interventions. The primary aim of this study was to examine the interrelationships between spatiotemporal gait characteristics at different walking speeds and a range of clinical and disease characteristics in individuals with FRDA. Thirteen people with FRDA walked along an 8.3 meter GAITRite® mat six times each at their preferred, fast and slow speeds. Relationships between spatiotemporal gait parameters, variability of spatiotemporal parameters and a range of clinical and disease characteristics were also examined. Significant correlations were found between spatiotemporal gait characteristics at each of the walking speeds and Friedreich Ataxia Rating Scale (FARS) score and disease duration. GAA1 repeat expansion positively correlated with double support percentage of the gait cycle in all speed conditions demonstrating a relationship between the genetic mutation and compensatory strategies for impaired dynamic balance. Age of onset negatively correlated with speed and cadence in the preferred and fast speeds, suggesting that earlier onset of FRDA has an effect on gait maturation. Heel-to-heel base of support positively correlated with the FARS lower limb coordination subscale in the preferred (r=0.602), fast (r=0.589) and slow (r=0.644) speed conditions, whilst the FARS upright stability subscale positively correlated with intra-individual variability of stride length in the preferred (r=0.579) and fast (r=0.660) speed conditions. There were no significant correlations between the FARS peripheral nervous system subscale and any spatiotemporal gait parameter measured. In all speed conditions, including at slow speed, there were correlations between a range of spatiotemporal gait characteristics and the timed 25 foot walk test, a well-established measure of gait mobility. This study reveals several interrelationships between spatiotemporal gait characteristics and a range of genetic and clinical markers of FRDA, suggesting earlier disease onset impairs the ability to compensate for stability challenges in gait. Moreover, these findings indicate that spatiotemporal gait parameters are a sensitive measure of gait decline in individuals with FRDA, and should be considered for inclusion in intervention studies whilst participants are still ambulant.

## OCT in Diagnosing ARSACS

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Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a rare neurodegenerative disorder caused by mutations in the SACS gene. Thickened retinal nerve fibres visible on fundoscopy have previously been described in these patients. However, thickening of the retinal nerve fibre layer as demonstrated by ocular coherence tomography appears to be a more sensitive and specific feature. To test this observation, we studied a cohort of patients with genetically confirmed hereditary ataxias (n=146) or idiopathic ataxias (n=45) presenting to a tertiary hospital specialist ataxia clinic in the UK. This included cases of Friedreich's ataxia (n=59), spinocerebellar ataxias (n=53), and other genetically confirmed ataxias (n=17). These were compared with known cases of ARSACS (n=17) and asymptomatic carriers of SACS mutations (n=21) by neurological and ophthalmological assessment, as well as time domain ocular coherence tomography. The cases included a novel SACS mutation (c.9404T>C). Most ARSACS patients were visually asymptomatic and had no previous history of ophthalmic complaints and normal or near normal visual test results. No ARSACS patient had visual symptoms directly attributable to the retinal changes. Twelve of the 17 patients with ARSACS (70.6%) had thickened retinal nerve fibres visible on fundoscopy, but all had thickening of the retinal nerve fibre layer on ocular coherence tomography. We propose a cut-off value of 119μm which provides a sensitivity of 100% and specificity of 99.4% amongst patients affected with ataxia. These findings show that ocular coherence tomography may be used to distinguish cases of ARSACS from other causes of ataxia but that visualization of thickened retinal fibres by direct fundoscopy is a less sensitive tool.

## Fatty acid oxidation is disrupted in the FRDA heart

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**Background:** Fatty Acid Oxidation (FAO) supplies ~70% of ATP demands in normal hearts, making β-oxidation of fatty acids a key metabolic activity to interrogate in the cardiomyopathy and heart failure of Friedreich's Ataxia (FRDA). We recently reported the novel finding that mitochondrial proteins in the heart from the FRDA animal model become heavily acetylated concurrent with cardiac hypertrophy and heart failure, and this is partially caused by inhibition of the NAD+-dependent SIRT3 deacetylase. We tested the hypothesis that mitochondrial dysfunction in FRDA leads to altered patterns of myocardial metabolic substrate utilization, and that mitochondrial protein expression can serve as a biomarker of disease severity. We predicted that the FRDA heart preferentially utilizes glucose due to mitochondrial dysfunction, thus placing FRDA patients at risk of death or morbidity with stressful events. We used cardiac Positron Emission Tomography (PET) to quantify glucose and fatty acid uptake in FRDA patient and control hearts, and in FRDA KO and control mouse hearts.

**Methods and Results:** Mice at 65 days of age underwent PET scan using 11C-Palmitate and 18FDG as tracers for FAO or glucose utilization respectively. FRDA KO mice (loss of FRDA gene in sarcomeric tissues driven by MCK-Cre transgene) were significantly lower ($p = 0.012$) in palmitate utilization rate (12.2/min, ±0.17, n=3) vs controls (13.7/min, ±0.20, n=2). In contrast, FRDA KO mice were significantly higher ($p = 0.047$) in glycolytic rate (34.7 ml/g·min, ±2.34, n=2) than controls (22.29 ml/g·min, ±1.55, n=2). Expression of mitochondrial proteins involved in FAO (PPARa, LCAD, MCAD) was not significantly different between groups, but hexokinase (glucose metabolism) protein expression was significantly higher in FRDA KO mouse heart ($p < 0.05$). In parallel with these studies, adult patients with FRDA (n=10), or adult controls (n=5), underwent echo, serum biomarker analysis, and PET scan with 11C-Palmitate and 18FDG. Partial multivariate analysis indicated that the FRDA patients had impaired ability to metabolize fatty acids and greater glucose utilization than controls. Interestingly, serum fatty acid binding protein 3 (cardiac FABP3) was significantly elevated in the FRDA patients ($p=0.001$), as were inflammatory markers IL-6 ($p=0.006$), ICAM-1 ($p=0.001$), and MIP-1a (0.013). Pearson's correlation coefficient between cTnI and FABP3 was 0.632. There was no significant difference in systolic function by echo, nor did GAA repeat correlate significantly vs controls for markers or PET scan.

**Conclusions:** FRDA patients appear to have impaired ability to utilize fatty acids for energy in heart, and this is confirmed using the FRDA KO mouse. Early biomarker analysis suggests that inflammatory and fatty acid biomarkers have significant associations, and may be informative in longitudinal FRDA population studies. The clinical implications of these findings are that FRDA patients may respond poorly to physiologic stress, and therefore require additional metabolic support.

## Biological and clinical characteristics of the European Friedreich Ataxia Consortium for Translational Studies (EFACTS): cross-sectional analysis of baseline data

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Background Friedreich ataxia (FRDA) is a rare autosomal recessive neurodegenerative disorder. We report cross-sectional baseline data to establish the biological and clinical characteristics for the first prospective FRDA pan-European database registry.
Methods within the European Friedreich Ataxia Consortium for Translational Studies (EFACTS), we assessed a large cohort of genetically confirmed FRDA patients. The primary outcome measure was the Scale for the Assessment and Rating of Ataxia (SARA), secondary outcome parameters were the Inventory of Non-Ataxia Signs (INAS), the performance-based coordination test Spinocerebellar Ataxia Functional Index (SCAFI), the neurocognitive verbal fluency and quality of life measures such as activities of daily living (ADL) and EQ-5D. The FRDA cohort was subdivided into three age-of-onset groups: early-onset (≤ 14 years), intermediate-onset (15 to 24 years), and late-onset (> 25 years), which were compared with respect to clinical characteristics and outcome measures. Linear regression analysis was used to estimate an annual rate of decline of clinical outcome measures based on disease duration. Findings We enrolled 592 genetically confirmed FRDA patients between 15-Sep-2010 and 30-Apr-2013 at eleven study sites. Age of onset was inversely correlated with the number of GAA repeats predicting, a 2.3-year-earlier onset rate with every 100 GAA repeats added on the smaller repeat allele. The commonest reported symptom at disease onset was postural instability (78%), followed by scoliosis (25%) and falls (20%). Subgroup analysis showed differences in almost all measures such as SARA, SCAFI, INAS as well as ADL, with more severe impairments in early-onset patients compared to intermediate or late onset. SARA, SCAFI and INAS strongly correlated with clinical and functional measures, while verbal fluency performance showed small to medium correlations. Regression analyses revealed an estimated annual rate of worsening in SARA in early-onset (1.04±0.13 points) and intermediate-onset patients (1.17±0.22 points), which was almost twice as high as in the late-onset group (0.56±0.10 points). This pattern was also observed for ADL. The most frequently reported non-neurological symptoms were cardiac and endocrinological impairment. Interpretation The cross-sectional EFACTS baseline analysis demonstrates that earlier disease onset in FRDA patients is associated with higher GAA repeat lengths and a more rapid disease progression. The differential estimated progression rates of ataxia symptoms related to age of onset and phenotype findings have implications for clinical trial designs, for which SARA and ADL might be in particular suitable to monitor disease progression in FRDA. Outlook The 12-month longitudinal data are currently analysed and will also be presented.
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## The views of individuals with, and parents of individuals with Friedreich ataxia regarding pre-symptomatic testing of minors

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Friedreich ataxia (FRDA) is an autosomal recessive neurodegenerative disorder characterised by variable age of onset, with no treatment proven to alter its natural history. Siblings of individuals with FRDA have a 1 in 4 risk of developing the condition, raising issues around genetic testing of asymptomatic minors. Currently, there is a lack of professional consensus and limited empirical evidence to support provision or refusal of pre-symptomatic testing for FRDA. This exploratory study aimed to ascertain the opinions of individuals with FRDA and parents of individuals with FRDA regarding pre-symptomatic testing of minors for the condition. A qualitative research approach using semi-structured interviews and thematic analysis was employed. Interviews with ten individuals with FRDA, and ten parents of individuals with FRDA were conducted, recorded, transcribed and analysed. Four key findings emerged. First, a number of arguments for and against testing minors were identified. Second, strong support existed from parents about the parental right to test their at-risk immature children, however individuals with FRDA were of mixed opinions. Third, most participants feel it is not the clinician's role to make a final decision about whether testing occurs. Finally, a specific issue of concern regarding testing was what and when to tell at-risk children about the test result. The findings from this study highlight the dilemma of how to manage the desires of some individuals and families affected by FRDA to access testing, when there is a lack of professional consensus due to differing opinions regarding autonomy, confidentiality and risk of harm. Further empirical research regarding the impact of such testing and the views of at-risk individuals and clinicians is required so an appropriate framework for dealing with this contentious issue is developed.

# Session 7: Clinical trials and trial design

## Saturday 28th March

*Chairs: Paola Giunti (University College London/UCL Hospital, UK) and Massimo Pandolfo (Université Libre de Bruxelles)*

Selected presentation

14:00 Richard Festenstein (Imperial College London, UK):
*Reversing FXN gene silencing in vivo in humans -towards a disease-modifying therapy?*

Invited speakers

14:20 Massimo Pandolfo (Université Libre de Bruxelles):
*Clinical trials in Friedreich's ataxia*

14:35 Interview with Pavel Balabanov (European Medicines Agency, London, UK)

Selected presentations

15:10 Paola Giunti (University College London, Institute of Neurology, UK):
*Can sensorimotor processing abnormalities explain or contribute to balance impairment in cerebellar disease?*

*1530 Break*

15:55 Zohar Argov (Bioblast Pharma, Chief Medical Officer, Israel):
*Double blind, randomized, controlled phase 3 trial of high dose IV trehalose (Cabaletta) for treatment of spinocerebellar ataxia 3 (SCA3)*

16:15 Robert Molinari (Retrotope Inc., US):
*An upcoming clinical trial testing the safety and efficacy of a stabilized polyunsaturated fatty acid in Friedreich's ataxia*

16:35 Colin Meyer (Reata Pharmaceuticals, Chief Medical Officer, US):
*Rationale and design of a clinical study of RTA 408 in patients with Friedreich's ataxia*

16:55 Gino Cortopassi (UC Davis School of Veterinary Medicine, US):
*Repurposed dyclonine for Friedreich's ataxia therapy*

17:15 General discussion

17:30 Close of conference

Selected presentation

## Reversing *FXN* gene silencing *in vivo* in humans -towards a disease-modifying therapy?

Vincenzo Libri[1], Cihangir Yandim[1], Sathiji Nagewasharan[1], Stavros Athanasopoulos[1], Naomi Loyse[1], Theona Natisvili[1], Pui Pik Law[1], Ping Kei Chan[1], Tariq Mohammad[1], Marta Mauri[1], Kin Tung Tam[1], James Leiper[1], Piper Sophie[1], Aravind Ramesh[1], Michael Parkinson[2], Les Huson[1], Paola Giunti[2], Richard Festenstein[1]

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**Background:** Friedreich's ataxia is a progressive degenerative disorder caused by deficiency of the frataxin protein. Expanded GAA repeats within intron 1 of the frataxin (FXN) gene lead to its heterochromatinisation and transcriptional silencing (Saveliev et al, Nature 2003). Preclinical studies have shown that the histone deacetylase inhibitor nicotinamide (vitamin B3) can remodel the pathological heterochromatin and upregulate expression of FXN (Chan et al, HMG, 2013). We aimed to assess the epigenetic and neurological effects and safety of high-dose nicotinamide in patients with Friedreich's ataxia. In this exploratory, open-label, dose-escalation study in the UK, male and female patients (aged 18 years or older) with Friedreich's ataxia were given single doses (phase 1) and repeated daily doses of 2—8 g oral nicotinamide for 5 days (phase 2) and 8 weeks (phase 3). Doses were gradually escalated during phases 1 and 2, with individual maximum tolerated doses used in phase 3. The primary outcome was the upregulation of frataxin expression. We also assessed the safety and tolerability of nicotinamide, used chromatin immunoprecipitation to investigate changes in chromatin structure at the FXN gene locus, and assessed the effect of nicotinamide treatment on clinical scales for ataxia (Libri et al Lancet 2014). In addition we have: 1) performed novel behaviourmetric analysis in order to develop non-invasive objective measures of both the existing clinical scales and activities of daily living; 2) developed a novel closed-loop coordination paradigm for fMRI analysis and 3) developed novel image techniques for assessing the dynamics of FXN expression.

**Results**: Nicotinamide was generally well tolerated; the main adverse event was nausea, which in most cases was mild, dose-related, and resolved spontaneously or after dose reduction, use of antinausea drugs, or both. Phase 1 showed a dose-response relation for proportional change in frataxin protein concentration from baseline to 8 h post-dose, which increased with increasing dose ($p=0{\cdot}0004$). Bayesian analysis predicted that 3·8 g would result in a 1·5-times increase and 7·5 g in a doubling of frataxin protein concentration. Phases 2 and 3 showed that daily dosing at 3·5—6 g resulted in a sustained and significant ($p<0{\cdot}0001$) upregulation of frataxin expression, which was accompanied by a reduction in heterochromatin modifications at the FXN locus. Clinical measures showed no significant changes. Nicotinamide was associated with a sustained improvement in frataxin concentrations towards those seen in asymptomatic carriers during 8 weeks of daily dosing. Further investigation of the long-term clinical benefits of nicotinamide and its ability to ameliorate frataxin deficiency in Friedreich's ataxia is warranted. To this end the novel behaviourmetric, fMRI and single cell analysis of FXN expression methodologies are being developed to accurately measure progression in this disease and response to therapy (MS in prep).

Invited Speaker: Massimo Pandolfo (University Libre de Bruxelles, Belgium)

## Clinical trials in Friedreich's ataxia.

Advances in understanding the pathogenesis of Friedreich's ataxia (FRDA) have led to insights that allowed the development of new therapeutic strategies for treating the disease. Some of these strategies aim to increase frataxin levels at least to those of asymptomatic heterozygous carriers. These include approaches to alleviate the epigenetic transcriptional silencing of the frataxin (FXN) gene by the expanded GAA repeats as well as approaches to induce frataxin expression regardless of the presence of the expansion mutation. Epigenetic silencing can be overcome by using histone deacetylase inhibitors (HDACi) acting on class I (benzamides) or class III HDACs (nicotinamide). A number of compounds upregulate frataxin expression in model systems, regardless of the presence of a GAA repeat. These include molecules as disparate as GLP-1 agonists, gamma interferon, diclonine, hEPO, and others, which very likely act by different mechanisms. Specific ubiquitin-competing molecules can prevent frataxin degradation in model systems. Frataxin protein replacement by using tat-frataxin and other modified frataxins is another currently investigated approach. Gene therapy with AAV vectors expressing frataxin has been shown to be effective in a mouse model of FRDA cardiomyopathy and is being intensively investigated for the neurological component of the disease.

Other potential therapies target pathogenic processes triggered by frataxin deficiency, as mitochondrial dysfunction, altered iron metabolism, and oxidative damage.

Some of the proposed therapeutics are new molecules, not previously used in humans, others are currently approved medications for other diseases that may have FRDA as a new indication. New drugs must go through a complete pre-clinical and clinical development.

Early efficacy studies must then be performed, following the golden standard of the randomized controlled trial (RCT). Unfortunately, so far there is no positive RCT in FRDA. While lack of efficacy of the tested therapeutics is a likely reason for missed efficacy endpoints, trial design is also critical. Efficacy endpoints may consist first in appropriately validated biomarkers, then in clinical parameters shown to be sensitive to disease progression. Choice of endpoints and trial design should aim to maximize efficiency, in order to identify those therapeutics that deserve further study in a reasonable time frame and without involving too many subjects. FRDA is a rare disease and it is relentlessly, but slowly progressive, so sample size and trial duration are critical. Ongoing large natural history studies in America, Australia and Europe (CCRN, EFACTS) are providing essential guidance for trial design. Harmonization of data collection is also very important to allow comparisons and meta-analyses. For this purpose, FRDA has been included in the NIH Common Data Element (CDE) project, leading to the identification and publication of sets of parameters to be collected in future clinical trials.

Interview with Pavel Balabanov (European Medicines Agency, UK)

**Biography**: I am a clinical neurologist by training, currently working as an EMA product lead (EPL) in CNS and Ophthalmology, Scientific and Regulatory Management Department, Human Medicines Evaluation Division of the European Medicines Agency.

In this role I am primary responsible for the provision of expert input in neurology for the department's core business in the Safety and Efficacy part related to pre- and post-authorization activities of centralized applications/marketing authorizations. My duties include also the maintenance of product oversight and follow-up on product related activities, addressing the life cycle development and interactions with external stakeholders, ensuring the quality of opinions, and providing support to the CNS working party and Scientific advisory groups and Committees.

I was previously employed in the Scientific Advice section of the Agency, where responsibilities included the provision of scientific advice and administrative support for the Scientific advice working party (SAWP), Committee for human medicinal products (CHMP) and other working parties and committees at the Agency.

I have a PhD in neurology on Pharmacoeconomics of anti-epileptic treatment, and am an assistant professor of neurology at the Medical University of Plovdiv, Bulgaria. My primary areas of interest include rare neuromuscular diseases, epilepsy, multiple sclerosis and stroke.

Selected presentation

## Can sensorimotor processing abnormalities explain or contribute to balance impairment in cerebellar disease?

Paola Giunti[3], Jonathan Marsden[2], Daniel Voyce[1], Brian Day[1], Lisa Bunn[1, 2]

*[1] Sobell Department of Motor Neuroscience and Movement Disorders, University College London, Institute of Neurology, UK, [2] School of Health Professions, Peninsula Allied Health Centre, Plymouth University, UK, [3] Department of Molecular Neuroscience, University College London, Institute of Neurology, UK*

**Background**: The cerebellum has the potential to participate in balance control as it receives considerable multi-sensory information, known to be important to balance control. Inherited types of cerebellar disease typically feature balance impairment. Balance impairment progresses over time alongside general disease severity but little is known concerning the mechanism through which this develops. Here we investigated spino-cerebellar ataxia type 6 (SCA6) to begin to explore any sensori-motor contribution to the degradation of balance control over time in a relatively well-defined and uncomplicated type of cerebellar disease.

**Objective**: To investigate whether balance impairments in SCA6 are associated with specific sensorimotor processing deficits experiments focused on the cerebellar functions of scaling, coordinate transformation and adaptation of balance responses.

**Methods**: Vestibular, visual and proprioceptive sensory channels were stimulated in isolation using galvanic vestibular stimulation, moving visual scenery and muscle vibration respectively in 16 subjects with spinocerebellar ataxia type 6 (SCA6) and 16 matched healthy controls. Two polarities of each stimulus type typically evoke balance responses of similar form in the forward and backward directions of healthy subjects. Balance responses were measured using whole body motion analysis. Baseline measures of normal body sway were recorded also using motion analysis and disease severity was assessed using the Scale for Assessment and Rating of Ataxia. Baseline measures were compared against response measures to sensory perturbations in order to specifically examine changes which scaled with disease progression.

**Results**: Faster measures of normal body sway confirmed balance impairment at baseline for the SCA6 group ($p=0.009$), which correlated with disease severity ($r=0.705$, $P<0.001$). The SCA6 group exhibited visually-evoked balance responses that were approximately three times larger than normal (backward, $p<0.001$; forward $p=0.005$) and correlated with disease severity ($r=0.543$, $p=0.03$). Vestibular and proprioceptive response magnitudes were not significantly different to healthy controls. Response direction and habituation properties were no different to controls for all three sensory modalities.

**Conclusion**: Sensory perturbations reveal a sensorimotor processing abnormality specific to response scaling to visual stimuli, which could significantly contribute towards balance impairment in cerebellar disease. The absence of decreases in gains of other sensorimotor channels suggests that this is not merely a re-weighting of sensory channels for balance control but rather a potential mechanism for instability. Cerebellar degeneration could disturb the scaling of balance responses evoked by visual motion through disinhibition of extracerebellar visuomotor centres.

## Double blind, randomized, controlled phase 3 trial of high dose IV trehalose (Cabaletta) for treatment of spinocerebellar ataxia 3 (SCA3)

Zohar Argov, Hagar Greif, Irit Gliko-Kabir, Dalia Megiddo

*Bioblast Pharma, Tel Aviv, Israel*

**Objective**: To describe the principles and design of a phase 3 trial of trehalose (Cabaletta) in SCA3 (Machado Joseph Disease)

**Background**: Trehalose is a disaccharide with protein stabilizing and autophagy enhancing properties. It showed efficacy in reducing abnormal protein aggregation in animal models of human poly A- and poly Q- mediated hereditary neurological disorders.

**Design and Methods**: 60 patients will be enrolled in an international multicenter study. Randomization will be in a 2:1 treatment: non treatment ratio. Patients will receive weekly infusion of 30 gr Cabaletta for 54 weeks. Main inclusion criteria: SCA3 (molecular confirmation) ambulant patients (stage 2) with disease duration <10 years. Primary endpoint: SARA scale. Secondary endpoints: NESSCA scale, 9HPT, 8MW, WHOQOL patient self report. An open label phase 2 study will precede the pivotal international trial.

**Results**: Safety profile of Cabaletta in our Oculopharyngeal Muscular Dystrophy (OPMD) trial, showed it to be well tolerated without associated safety events, enabled opening the Israeli phase 2 trial in October 2014. Pharmacokinetic data from the OPMD trial showed that the levels of plasma trehalose after a single 30 gr dose reached the expected concentrations determined in the animal studies as necessary for its intracellular activity (max. levels of 1000-2000 mg/mL after 1 hour), and were retained up to 5 hours post injection. A subtle increase (mean= 5 mg%) in plasma glucose concentrations was observed 1 hour after administration, without increase in insulin levels. Short term glycosuria was recorded, due to putative kidney trehalase activity.

**Conclusions**: Based on these preliminary findings, high dose IV trehalose (Cabaletta) is safe and tolerable in humans. The human pharmacokinetic profile will enable trehalose to reach the target affected cells. The SCA3 phase 2/3 study will be launched in 2015. More disorders with similar PolyA/Poly Q genotypic changes may be suitable for such trials.

## An upcoming clinical trial testing the safety and efficacy of a stabilized polyunsaturated fatty acid in Friedreich's ataxia

Robert Molinari[1], MIkhail Shchepinov[1], Maria Cotticelli[2], Robert Wilson[2], Ann Murphy[3], Alexei Andreev[3]

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Polyunsaturated fatty acids (PUFAs) are susceptible to an accelerating damage cascade from an autocatalytic, free radical chain reaction. Damaged lipid end products (e.g. 4-hydroxy-nonenal and others) from this process have been associated with mitochondrial dysfunction and a host of age-related degenerative diseases, including Friedreich Ataxia (FRDA). Cells in multiple models of FRDA, when treated with a stabilized lipid mimetic of the normal dietary PUFAs in mitochondrial membranes, show stunning reversal of lipid peroxidation damage, increased cell viability, and improved mitochondrial function. The mechanism of action of the drug, a stabilized form of the essential fat linoleic acid, is believed to be down-regulation of PUFA autoxidation initiated by hydrogen abstraction from susceptible, bis-allylic sites of mitochondrial membrane PUFAs. Replacement of the bis-allylic hydrogen atoms with deuterium atoms (D-PUFAs) arrests PUFA autoxidation in vitro and in vivo due to the kinetic isotope effect. Unlike antioxidants, which are typically consumed as they quench lipid peroxidaiton products, D-PUFAs are not used up in the process of inhibiting lipid peroxidation, and don't suffer from the distribution and diffusion limitations of antioxidant approaches.

Surprisingly, cells from yeast, murine, and human (primary FRDA patient cells) treated with a mixture of approximately only 20% isotope-reinforced D-PUFA in a background of normal PUFAs are fully protected from lipid autoxidation-mediated cell killing. The findings also show mitigation of mitochondrial dysfunction and increased cell viability. As a minor perturbation on naturally occurring GRAS fats, D-PUFA drugs enjoy all the active transport in an out of tissues and mitochondria that evolved over decades to esnure critical PUFA molecules were replaced when damaged, and were granted an accelerated pathway into human testing by the US FDA. A trial in the orphan neurodegenerative disease, Friedreich Ataxia, is planned. Orally fed rodent models in other degenerative diseases and PK studies of the drug confirm efficacy in difficult to reach brain and retina tissues, and IND-enabling toxicity studies showed no signs of drug related adverse findings in any parameter tested.

The planned Phase 1b/2a trial in 33 patients dosed for 6 months is expected to start in early 2015, will include an ascending dose safety study, and will measure FARS and multiple other FRDA disease readouts.

## Rationale and design of a clinical study of RTA 408 in patients with Friedreich's ataxia

Colin Meyer[1], Angie Goldsberry[1], Megan O'Grady[1], Jen Farmer[2], David Lynch[3]

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RTA 408 is a semi-synthetic triterpenoid that potently induces nuclear factor erythroid-derived 2-related factor 2 (Nrf2) and suppresses NF-κB at low nanomolar concentrations. Through modulation of these transcription factors, RTA 408 regulates multiple genes that play both direct and indirect roles in the production of cellular energy within the mitochondria. Genetic induction of Nrf2, as well as pharmacologic induction with RTA 408 and related analogs, has been shown to increase mitochondrial function in preclinical and *ex vivo* systems, by increasing reducing equivalents, oxygen consumption, and ATP production.

A hallmark of Friedreich's ataxia is impairment of antioxidative defense mechanisms, which play a major role in disease progression. Studies have demonstrated that Nrf2 signaling is grossly impaired in patients with Friedreich's ataxia and likely contributes to oxidative stress and reduced ATP production. Clinically, these effects manifest as reduced exercise capacity, visual function, energy levels, and quality of life. Therefore, the ability of RTA 408 to activate Nrf2 and induce antioxidant target genes is hypothesized to affect these abnormal biochemical and clinical deficits in patients with Friedreich's ataxia.

This phase 2 study of the safety, efficacy, and pharmacodynamics of RTA 408 in the treatment of Friedreich's Ataxia (MOXIe; NCT02255435) is a two-part study. The primary efficacy endpoint is the time-averaged effect on peak work during maximal exercise testing following 12 weeks of treatment with RTA 408 as compared to placebo. The study will also explore changes in the modified Friedreich's ataxia rating scale (FARS) score and changes in patient reported outcomes.

The first part of this study is a randomized, placebo-controlled, double-blind, dose-escalation study to evaluate the safety, efficacy, pharmacokinetics and pharmacodynamics of RTA 408 at 2.5 mg, 5 mg, and 10 mg in 16 total patients. The second part of this study will be a randomized, placebo-controlled, double-blind, parallel study to evaluate the safety, efficacy and pharmacodynamics of up to two dose levels of RTA 408 in 24-36 patients with Friedreich's ataxia. Patients in Part 2 will be randomized 1:1:1 to receive RTA 408 2.5 mg, RTA 408 10 mg, or placebo (n=8-12 per treatment group) and will be stratified by peak work at baseline. All qualified patients enrolled in the study will follow similar schedules of assessments and study drug administration. Patients will self-administer study treatment once daily for 12 weeks. A data safety monitoring board will perform monthly reviews of data for safety throughout the study.

## Repurposed dyclonine for Friedreich's ataxia therapy

Gino Cortopassi, Sunil Sahdeo

*University of California, United States*

There is currently no approved therapy for Friedreich's ataxia in the US. Because there is often inertia for large pharma to develop drugs for rare disease, we screened a library of FDA-approved compounds to identify drugs which might be effective in Friedreich's ataxia. From microarray and RNAseq of DRG neurons, defects in iron-sulfur and antioxidant transcripts and enzymes was identified, associated with mitochondrial antioxidant activity, eg. thioredoxin reductase. Thus, Friedreich's cells were screened with multiple poisons of thiol antioxidant systems, and sensitivity to diamide was observed. Using diamide sensitivity as a screen, 1600 compounds in clinical use were screened for protection from diamide. Of these 1600, 40 were protective. Of the 40, 10 induced frataxin expression in Friedreich's patient cells. We identified the topical anesthetic dyclonine as protective. Dyclonine increased FXN transcript and FXN protein dose-dependently in FA cells and brains of animal models. Dyclonine also rescued FXN-dependent enzyme deficiencies in the iron-sulfur enzymes, aconitase and succinate dehydrogenase. Dyclonine induces the Nrf2 [nuclear factor (erythroid-derived 2)-like 2] transcription factor, which we show binds an upstream response element in the FXN locus. Additionally, dyclonine also inhibited the activity of histone methyltransferase G9a, known to methylate histone H3K9 to silence FA chromatin. Chronic dosing in a FA mouse model prevented a performance decline in balance beam studies. A human clinical proof-of-concept study was completed in eight FA patients dosed twice daily using a 1% dyclonine rinse for 1 week. Six of the eight patients showed an increase in buccal cell FXN levels, and fold induction was significantly correlated with disease severity. Dyclonine represents a novel therapeutic strategy that can potentially be repurposed for the treatment of FA.

# Poster Session 1: New genes and developments in diagnosis of the ataxias

**Wednesday 25th March 18:30-20:15**

**P001**

## Screening study of SCA-negative ataxia patients for presence of Friedreich's ataxia trinucleotide expansion mutation

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**Background**: Patients presenting with ataxia are routinely tested for a panel of expansion mutations in genes for the spinocerebellar ataxias (SCA1, 2, 3, 6, 7, 12 and 17). These patients predominantly have late disease onset and a dominant pattern of inheritance. By contrast, Friedreich's ataxia (FRDA) usually presents before age 25 and is recessively inherited. Therefore, a genetic test for FRDA is not routinely included in the diagnostic screening package for atypical ataxia patients, many of whom are also negative for all the routine screens and are thus lacking a formal genetic diagnosis. Since the discovery of the causative frataxin (FXN) gene in 1996, atypical and later onset cases of FRDA have been identified. This information led us to question whether the root cause of many of these idiopathic ataxia cases could be the FRDA mutation.
**Objective**: To screen over 2000 idiopathic ataxia patients for the FRDA mutation, and observe the genotype-phenotype correlation in any FRDA-positive cases. This is the largest population size of ataxic patients ever tested for FRDA. Our findings will allow us to decide in a more evidence-based manner whether FRDA testing should be included in the routine genetic screening package for ataxia patients of unknown etiology.
**Methods**: Molecular diagnostic tests can be used to detect the GAA expansion mutations and thus diagnose FRDA. Triplet-primed PCR was first used to detect the presence of the expansion. For samples where the expansion was discovered, long range PCR was performed to determine whether the subject was a carrier or homozygote for the GAA expansion, and thus whether the patient was FRDA-positive. The relevant clinical information was obtained to observe the genotype-phenotype correlation in positive cases.
**Results**: Out of 2021 idiopathic ataxia patients screened thus far, 45 have been found to carry the FRDA expansion on at least one allele by the TP-PCR method. Of these samples, 20 have so far been screened by Long Range PCR, with four FRDA positives confirmed, eleven FRDA carriers confirmed and five samples unconfirmed. The other 25 patients are still under investigation. Carriers will be screened for point mutations: if again negative, they will be tested for exonic deletions.
**Conclusion**: These results provide more evidence that patients who present with features typical of the SCAs, only rarely represent atypical presentations of FRDA. These results should be added to the guidelines for the genetic testing and counselling of ataxia patients.

**P002**

## Characterisation of the genetic background of childhood ataxias in Finland

Christopher Carroll[1], Erika Ignatius[1, 2], Pirjo Isohanni[1, 2], Eino Palin[1, 2], Anu Suomalainen[1, 3], Tuula Lönnqvist[2]

*[1] Research Programs Unit, Molecular Neurology, Biomedicum-Helsinki, University of Helsinki, Finland, [2] Department of Pediatric Neurology, Children's Hospital, Helsinki University Central Hospital, Finland, [3] Department of Neurology, Helsinki University Central Hospital, Finland*

Inherited ataxias comprise a genetically heterogeneous group of diseases with mutations in more than 60 genes having been linked to ataxia. The molecular background of genetic diseases in Finland is unique, which is owed to the genetic bottleneck that resulted from the small founder population that settled in Finland. The bottleneck led to enrichment of rare variants and accounts for the Finnish Disease Heritage. In Finland, the most common recessive ataxias are caused by variants enriched in Finns, including mutations in *POLG* causing mitochondrial inherited recessive ataxia syndrome and *C10orf2* causing infantile-onset spinocerebellar ataxia (IOSCA). However, most childhood ataxias in Finland remain without a molecular diagnosis. We report here the results of a whole-exome sequencing approach of a cohort of 30 Finnish patients with ataxia. DNA was sequenced at a minimum depth of 10-fold for more than 95% of the exome. We customized an analysis pipeline, and prioritized rare variants using a unique population variant data resource over 3300 Finnish exomes (Sequencing Initiative Suomi) which is integrated in the Exome Aggregation Consortium (ExAC) database. Data analysis is ongoing and in 15 patients investigated so far a candidate has been prioritized in four patients (27%). Prioritised candidates are being validated by segregation analysis of variants in family samples. We conclude that whole-exome sequencing is an effective method for the molecular diagnosis of childhood ataxias, with high potential to lead to specific diagnosis early in the diagnostic workup.

**P003**

## Clinical and genetic characterization of the first SCA5 Italian family with a novel *SPTBN2* gene mutation

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Spinocerebellar ataxias with autosomal dominant inheritance (SCAs) are a group of clinically and genetically heterogeneous forms. To date, more than 30 different loci have been identified. The most commonly identified conditions worldwide are SCA1, SCA2, SCA3, and SCA6, which are caused by CAG (polyglutamine) expansions in their specific genes, and account for approximately 50% of the familial cases. Conventional mutations are identified in other rare autosomal dominant ataxias. Among these, SCA5 is caused by mutations in the beta-spectrin, nonerythrocytic, 2 gene (beta-III spectrin, *SPTBN2*).

To date, three in-frame deletions and two missense mutations in the *SPTBN2* gene have been reported in SCA5 families originating from the United States, Europe, and Japan. In these families, age at onset ranges from the 3rd to the 5th decade and the clinical presentation is characterized by a slowly progressive cerebellar syndrome. A complex phenotype has been described in one child with no family history and a heterozygous missense mutation with unclear pathogenic effect. More recently, homozygous nonsense mutations have been identified in two consanguineous families with very early-onset autosomal recessive ataxia associated with psychomotor delay (SCAR14).

In this study, we report a novel three-generation Italian family with five ascertained affected members. The clinical phenotype is characterized by adult-onset slowly progressive cerebellar ataxia, mild dysarthria and limb ataxia, dysphagia, increased DTRs. The proband was a 62-year-old woman who presented gait ataxia since the age of 36. At clinical examination, she had mild dysarthria, dysphagia, and incoordination of the upper and lower limbs. She also had gait ataxia, and walk was possible only with unilateral support. Knee tendon reflexes were slightly increased bilaterally, but Babinski's sign was negative. Cognition was normal and no sensory abnormalities were observed. The majority of the affected subjects of the family also present slowly progressive cerebellar ataxia with urinary sphincter disturbance. At MRI investigation, cerebellar atrophy with normal brainstem was observed in all cases. Genetic screening, performed by high-throughput sequencing, allowed the identification of a novel mutation, characterized by an in-frame insertion of 24bp in the third spectrin domainof the *SPTBN2* gene (c.1896_1919dup). The mutation segregated with the clinical phenotype and was not present in 188 control subjetcs of the same ethnic origin. This is the first Italian family with SCA5 genotype. The clinical features are similar to those reported in other SCA5 families, whose affected members also present adult-onset slowly-progressive pure cerebellar ataxia. (Supported by a grant from the Italian Ministry of Health to F.T.)

**P004**

## Exome sequencing in cerebellar ataxias illustrates the inter-relationship between diagnostics and novel gene discovery

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Inherited cerebellar ataxias have highly heterogeneous clinical phenotypes, patterns of inheritance and types of mutation, which creates significant challenges for developing diagnostic services. Expansion of these services also relies on the discovery of new genes requiring significant research resources.

Nemeth et al., 2013 reported a pilot study using targeted capture followed by next generation sequencing aimed at clinical diagnostics for ataxias and this is now offered as a diagnostic service by the Oxford Regional Genetics Laboratories, UK. The preliminary data obtained from this service is presented separately by Williams et al.

The initial pilot study has been followed by exome sequencing in 18 trios or siblings with cerebellar ataxia. 10 of the cases were pre-screened using the targeted panel and 8 were not.

Exome capture was performed using the SureSelect Human All Exon kit v5 (Agilent Technologies) and 100 bp paired-end sequencing was performed on the Illumina Hiseq 2000 platform. At least 92% of the target region was covered 20X. Sequences were analysed using standard and customised bioinformatics tools at CGAT and filtered using dominant, recessive and *de novo* models. Variants were visually inspected using the Integrative Genomics Viewer (IGV) and validated by Sanger sequencing.

All 8 of the families who were not pre-screened were found to have mutations in known ataxia genes, including *SPTBN2*, *ITPR1, GOSR2* and *SPG7* but some of the clinical features, age of onset and patterns of inheritance (eg *de novo* dominant) were very unusual. Mutations in some genes were novel and have required functional validation on a research basis.

6/10 families who were negative on pre-screening were found to have mutations in novel candidate genes, which are being genetically and functionally validated. These genes encode proteins which affect a variety of pathways involved in cerebellar development and will be presented in further detail. The other 4 families did not have any good candidate mutations identified.

Although exome sequencing widens the numbers of genes analysed and was effective in identifying mutations, it suffers from lower depth of sequence coverage which may explain the lack of a molecular diagnosis in some cases. Therefore it may ultimately be replaced by genome sequencing. In addition, the sequencing costs were higher and time to diagnosis was much longer than using the targeted capture approach.

In conclusion, novel gene discovery is aided by diagnostic services and new genes identified can be added to diagnostic panels once further validation is completed.

Until genome sequencing combined with targeted analysis becomes financially viable in its own right, a streamlined and cost effective approach for both clinical diagnostics and novel gene discovery is likely to involve targeted capture on a clinical service basis followed by exome/genome sequencing in negative cases.

**P005**

## Exome sequencing in unexplained ataxia

Tania Smertenko[1], Angela Pyle[1], Rita Horvath[1], David Bargiela[1], Helen Griffin[1], Jennifer Duff[1], Kim Bartlett[2], Konstantinos Douroudis[2], Gerald Pfeffer[1], Mauro Santibanez-Koref[1], Gail Eglon[1], Patrick Yu-Wai-Man[1], Venkateswaran Ramesh[3], Patrick Chinnery[1]

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Inherited ataxias are clinically and genetically heterogeneous, and a molecular diagnosis is not possible in the most patients. Having excluding common sporadic and inherited causes, we used exome sequencing to define the likely molecular diagnosis in 11 of 22 families. Three families had novel compound SACS mutations, each found in two affected siblings, including a full deletion of SACS detected from exome coverage. Known compound heterozygous SPG7 mutations were found in three affected individuals from one family with no spasticity. Three siblings presenting with adult onset ataxia had compound heterozygous mutations in NPC1, confirmed by subsequent oxysterol analysis. One had de novo compound heterozygous mutations in ZFYE26 (SPG15). Likely de novo dominant TUBB4A mutations were found in two families. One showed varying degrees of mosaicism in the mildly affected mother and heterozygosity in the severely affected offspring. A known dominant SLC1A3 mutation segregated with ataxia in three members of a family. Previously described compound heterozygous WFS1 mutations (p.Lys193Gln and p.Arg456His) were identified in one family. Finally, a known dominant KCNC3 mutation segregated with ataxia in four members of a three-generation autosomal dominant pedigree, making this family the 6th described to date with a mutation in this gene. The diagnostic yield was the same in both young and older-onset patients, including sporadic cases. This demonstrates the extreme genetic heterogeneity, but shows the impact of exome sequencing in a group notoriously difficult to diagnose genetically.

**P006**

## Bridging the gap – Is there need for "Ataxia Africa"?

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The hereditary ataxias are characterised by progressive neurodegeneration, leading to permanent disability. This has a significant impact on the quality of life of the affected patients, family members and caregivers. Furthermore, palliative care and management of these individuals comes at a significant cost. The global epidemiology of the hereditary ataxias is largely unknown, but recent meta-analyses have lead to the estimation that an average of 2.7 per 105 individuals are affected with autosomal dominant hereditary ataxia worldwide. Given the current population estimate of 1.11 billion, approximately 30 000 individuals on the African continent may be affected. However, reports on the prevalence of the inherited ataxias in Africa have been scarce. To our knowledge, the National Health Laboratory Service (NHLS) at Groote Schuur Hospital in Cape Town is the only state laboratory in Africa offering a molecular diagnostic service for the inherited ataxias.

The work presented in this study serves as the most recent update on the spectrum and prevalence of the inherited ataxias in South Africa, and represents the largest patient cohort described on the African continent. A total of 313 individuals from 215 families have received a molecular diagnosis of Spinocerebellar ataxia types 1, 2, 3, 6 or 7 over a 26-year period of testing. Whilst SCA3 is typically the most common of the SCAs worldwide, an over-representation of SCA1 and SCA7 has been observed in South Africa. Previous studies have identified conserved haplotypes within SCA1 patients from the mixed ancestry sub-population, and Black African SCA7 patients, indicative of a founder effect within each of these groups. Recent investigations have shown that two Zambian families and a Namibian family share the South African SCA7 haplotype, representing the first report of affected families from additional Sub-Saharan countries, and indicating that the founder effect extends beyond South African borders.

Future work will focus on increasing the awareness of the inherited ataxias amongst the clinical community in Africa, in order to improve the level of patient referrals for molecular testing, and to investigate the extent, distribution and frequency of the inherited ataxias in Africa. This would ultimately benefit a large number of individuals, since a confirmed molecular diagnosis can enable more appropriate clinical management, and family members can be counselled with regard to their own risk, as well as the risk for current and future offspring. Pilot studies will be implemented to determine other types of inherited ataxia in African populations. It is anticipated that improved interactions between clinicians, diagnostic laboratories and researchers may ultimately lead to better management, counselling, care and outcomes for ataxia patients on the African continent.

## P007

### Facing the diagnostic challenge: a suspicion index allows identification of Niemann-Pick disease Type C among patients with complex early-onset ataxias

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**Objectives:** Early-onset ataxias (EOAs) are a highly heterogeneous group of degenerative and metabolic diseases, predominantly caused by recessive mutations in genes with pleiotropic, multisystemic manifestations. The range of disorders with identified genetic and phenotypic heterogeneity has been expanding rapidly in recent years, complicating and prolonging the diagnostic process in patients presenting with unclear EOA. Confirming a genetic diagnosis is particularly important in EOAs where an evidence-based drug treatment is available for the underlying disease. Niemann-Pick disease Type C (NP-C) is a recessive lysosomal lipid storage disorder, presenting with EOA as a cardinal feature, for which treatment is available. A suspicion index (SI) was developed as a screening tool to aid early detection and diagnosis of NP-C in patients with complex heterogeneous symptomology, but its predictive value has not yet been investigated in EOA populations, where specificity might be much lower.

**Methods:** A consecutive series of EOA cases (N=57), negative for *NPC* mutations (non-NP-C ataxia cases), was collected at the ataxia outpatient clinic in Tübingen, Germany, and compared with a multicentre cohort of existing EOA cases (N=47) with a diagnosis of NP-C, confirmed by filipin staining and genetic testing (NP-C ataxia cases). Both cohorts were systematically phenotyped. NP-C signs and symptoms were categorised into visceral, neurological, or psychiatric domains, as structured within the NP-C SI, and summarised by descriptive statistics. Differences between NP-C ataxia cases and non-NP-C ataxia cases were assessed by Chi-Square test, or Fisher's exact test as appropriate. Using the NP-C SI, a score was calculated for all patients and the discriminatory performance assessed.

**Results:** A substantial number of non-NP-C ataxia cases yielded a moderate (40–69 points; n=11/57=19%) or high (≥70 points; n=3/57=5%) NP-C SI score, indicative of the multisystemic neurological phenotype, characteristic of many EOAs. Correspondingly, some items within the NP-C SI lost their discriminatory value for differentiating NP-C cases from non-NP-C cases when applied in an EOA population, e.g. ataxia, dysarthria/dysphagia, myoclonus, and spasticity. However, many other items supported discrimination of NP-C cases from non-NP-C cases within this population, e.g. neonatal jaundice/cholestasis, splenomegaly, vertical supranuclear gaze palsy, gelastic cataplexy, psychotic symptoms, and pre-senile cognitive decline/dementia. Overall, the NP-C SI had excellent discriminatory performance, differentiating NP-C ataxia cases from non-NP-C ataxia cases. The cutoff points as originally defined by the SI also help to grade suspicion of NP-C in patients with EOA.

**Conclusions:** Although some items lost their discriminatory value, the NP-C SI yielded excellent discriminatory performance in these patient populations with degenerative EOA. In the field of genetically and phenotypically complex EOAs, the NP-C SI will reliably help to identify those patients with EOA who may warrant further investigation for NP-C.

**P008**

## Efficient molecular diagnosis of autosomal recessive ataxia

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*The Cyprus Institute of Neurology and Genetics, Cyprus*

Autosomal recessive ataxias are characterized by vast genetic and phenotypic heterogeneity with more than 40 different genes thus far identified. Therefore, molecular genetic diagnosis becomes challenging and many families remain undiagnosed. Recent advances in the NGS technologies provide a strong tool for the efficient diagnosis of patients and the discovery of new ataxia genes. We hereby present results of the NGS analysis of nine Cypriot autosomal recessive ataxia families previously excluded from the most frequent ataxia genes/loci.

Whole-exome sequencing (WES) was performed on the proband of each family. For one of these families, whole-genome homozygosity mapping preceded the WES analysis thus enabling the analysis of the obtained WES data to be focused on a specific chromosomal region. In order to conclude on the causative mutations, candidate variants were validated by Sanger sequencing and segregation analysis, followed by screening normal control Cypriot chromosomes.

This investigation resulted in the identification of six different mutations in six different genes, in six of the families under study. Overall, a novel homozygous missense mutation in the GBA2 gene, a novel homozygous missense mutation in the SPG7 gene, a homozygous two base pair deletion that leads to a frameshift in the SPG11 gene, a novel homozygous nonsense mutation in the ANO10 gene, a novel homozygous missense mutation in the CLN6 gene and a novel homozygous missense mutation in a novel gene that is currently under further investigation, were identified. For the remaining three families the candidate variants obtained by the WES data are still under investigation.

Genotype-phenotype correlation enabled confirmation of the genetic findings since prominent symptoms of the Cypriot patients are in the majority consistent with those reported in other populations for patients with mutations in the corresponding genes.

In conclusion, NGS analysis enabled immediate molecular diagnosis of six additional Cypriot families. The robustness of WES towards the efficient molecular diagnosis of patients and the identification of novel mutations in rare diseases such as ataxia is further proved. This study expands the spectrum of the autosomal recessive ataxia mutations in the Cypriot population and proves the existence of marked genetic heterogeneity within the Cypriot ataxia families.

**P009**

## Diagnosis of hereditary ataxias by targeted next generation sequencing

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The hereditary ataxias are a heterogeneous group of conditions in terms of associated clinical features, age of onset, progressive nature and, most importantly, genetic basis. The most common of the ataxias (SCAs 1-3,6,7,17 and Friedreich's Ataxia) are caused by trinucleotide repeat expansions which can be readily detected by standard PCR-based techniques. By contrast, individuals with rarer forms often have a long diagnostic odyssey involving the sequential screening of individual candidate genes by Sanger sequencing. This approach is both costly and time consuming and is further complicated by the limited availability of diagnostic gene sequencing for many of the known candidates.

Based on data from Nemeth et al., 2013, the Oxford Medical Genetics Laboratories have introduced a UKGTN approved Next Generation Sequencing (NGS) service covering 91 ataxia associated genes, to facilitate molecular diagnoses in these patients. The included genes cover the full range of Mendelian inheritance models and a wide range of clinical conditions including adult and childhood onset ataxias, a number of episodic ataxias, spinocerebellar ataxias, and pontocerebellar hypoplasia.

The coding regions and intron-exon boundaries of these genes are targeted using Agilent's Haloplex™ Targeted Enrichment technology and sequenced on the Illumina Miseq platform allowing us to achieve high depth of coverage across the panel (average >97% of target regions covered at a depth of 20x or higher). The flexibility of this capture system has allowed us to incrementally increase the size of our panel from a starting point of 43-genes through 57- and 77- to the current 91-gene design. Moreover, an in-house method for analysing depth of coverage has allowed us to detect putative copy-number variants in genes for which commercial MLPA kits are not available.

To date, approximately 90-patients have been taken through the iterations of the panel, with a molecular diagnosis being made in 14 (~15%). A series of case studies will be presented highlighting the clinical utility of our approach along with a discussion of our NGS strategy.

# Poster Session 2: Genetic and molecular mechanisms of the ataxias

**Wednesday 25th March 18:30-20:15**

**P010**

## A plant model for understanding the genetic and molecular mechanisms underlying triplet expansion disorders

Sureshkumar Balasubramanian

*Monash University, Australia*

Triplet repeat expansions underlie several neuronal disorders and until recently they have been discovered only in humans. We have reported a GAA/TTC triplet repeat expansion associated genetic defect in a population of the model plant Arabidopsis thaliana and experimentally demonstrated that the repeat expansion causes the down regulation of the IIL1 gene, which harbours the expansion, which leads to a conditional growth defect (Sureshkumar et al, Science, 2009). Having discovered the first example for a triplet expansion associated genetic defect outside humans, we have exploited this system to ask questions, which are not easily feasible in the human systems. I will present our system, discuss the similarities with Friedreich's ataxia, explain the commonalities and demonstrate how the plant system can be an excellent complement to ongoing efforts to manage repeat expansion associated genetic defects.

**P011**

## Defining the pathogenic role of mitochondrial DNA mutations in neuronal degeneration in Friedreich's ataxia

Angela Bhalla, Marek Napierala

*The University of Alabama at Birmingham, US*

Friedreich's ataxia (FA) is an autosomal recessive neurodegenerative disorder elicited by epigenetic silencing of a GAA trinucleotide repeat expansion located within the first intron of the frataxin gene (FXN). FXN functions as a nuclear-encoded mitochondrial protein necessary for iron-sulfur cluster biosynthesis. Its deficiency has multiple effects within the cell including mitochondrial iron overload and increased sensitivity to reactive oxygen species (ROS). Current research suggests ROS contribute to the molecular pathogenesis of FA by causing oxidative stress, however, the consequences of ROS on the integrity of the mitochondrial genome remain unclear. Previous studies from other laboratories showed mitochondrial DNA damage in yeast expressing low levels of human frataxin and peripheral blood cells of FA patients. Building upon this finding, we hypothesize affected FA patient cells accumulate ROS within their mitochondria, causing increased mitochondrial DNA (mtDNA) mutations that have a pathogenic effect on the cell by impairing mitochondrial function, ultimately leading to neurodegeneration in FA cells.

In this study, we present detailed bioinformatic analyses of the mutation spectrum such as insertions and deletions and the transition to transversion ratio, and the frequency of mtDNA mutations in different cellular FA models. Specifically, we determined the mtDNA mutation frequency by deep sequencing the mitochondrial genomes of FA patient and unaffected control primary fibroblasts, cerebrum and cerebellum tissues, and iPSC-derived neurons. In parallel, we used qPCR to measure mtDNA damage and determined mitochondrial function in FA patient and unaffected fibroblasts and iPSC-derived neurons. Lastly, we utilized an isogenic zinc-finger nuclease (ZFN) corrected FA cell line generated in our laboratory to determine whether increased frataxin expression will have a protective effect against mtDNA mutations and improve mitochondrial function. Our qPCR analyses show increased mtDNA damage in FA fibroblasts compared to unaffected control fibroblasts. In addition, deep sequencing results from the mitochondrial genomes of fibroblasts indicate that the mtDNA mutation frequency is increased within the mitochondrial genomes of FA affected individuals compared to unaffected controls. Importantly, results of this study will provide insights into the contribution of pathogenic mtDNA mutations to mitochondrial phenotype in FA fibroblasts and neuronal cells, as well as their effect on the cellular and molecular phenotype of FA. We believe these results will advance our understanding of the molecular mechanism of neurodegeneration in FA and direct the development of novel disease therapies.

**P012**

## SCA36: molecular analysis, expansion size determination, and clinical features in Italian patients

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Autosomal dominant spinocerebellar ataxias (SCA) are a heterogeneous group of neurological disorders characterized by cerebellar dysfunction mostly due to Purkinje cell degeneration. Recently, a novel form of spinocerebellar ataxia (SCA36) with motor neuron involvement was described in Japanese and Galician patients. It is caused by a GGCCTG repeat expansion in intron 1 of *NOP56* gene. This gene encodes a component of the ribonucleoprotein complex and plays a role in transcription and splicing. *Objectives*: To screen a large cohort of Italian unrelated patients with familiar (n=150) or sporadic (n=276) spinocerebellar ataxia for a large (7-21 kb) GGCCTG repeat expansion in *NOP56* intron 1. All patients were negative for the common SCA1 and SCA2 mutations. *Methods*: Screening for the *NOP56* GGCCTG repeat expansion was performed by fluorescent triplet repeat-primed PCR (TP-PCR) analysis, using three primers including one fluorescent-dye-conjugated forward primer, a first reverse primer consisting of 4 repeat units and a 5' anchor tail, and a second reverse anchor primer. *Results*: *NOP56* intron 1 repeat expansion was detected in 10 probands from 7 different unrelated Italian families, 6 with familial ataxia. Inheritance was clearly dominant in 4 families. In other 2 families, 2 siblings were affected, while only 1 subject was affected in the last family. In order to confirm the expansion and to determine the number of the repeats, we optimized a protocol for the detection and sizing of SCA36 expansion by nonradioactive Southern blot hybridization. Analysis of 3 patients revealed expanded alleles between 930 and 3500 repeats. Normal alleles in the SCA populations ranged from 6 to 14 repeats, with the 9-repeat allele being the most frequent. Interestingly, all probands originated from a relatively small area in Central Italy, suggesting a common ancestor and a founder effect for this mutation, similarly to what observed in the Japanese and Galician populations. *Conclusions*: SCA36 accounts for approximately 4% of Italian autosomal dominant SCA families negative for the common SCA mutations. A nonradioactive Southern blot hybridization method was developed to confirm the expansion. Clinically, mutated patients presented with slow progressive gait ataxia with late onset (40-60 yrs) with pyramidal signs, eye movement abnormalities and, in some cases, motor neuron involvement with tongue atrophy. Neuroimaging revealed prominent cerebellar atrophy affecting the vermis, with minor involvement of cerebellar hemispheres and brainstem in later stages.

**P013**

## MLH3 isoform 2 does not make the cut in Friedreich ataxia GAA•TTC repeat somatic expansion

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Friedreich ataxia (FRDA) is a progressive neurodegenerative disorder caused by GAA·TTC repeat expansion in the first intron of the frataxin (FXN) gene and is the most common inherited ataxia. Disease severity correlates to the length of the expanded repeats and the consequent reduction of FXN mRNA. We aim to refine our knowledge of the somatic repeat expansion process that causes FRDA. Previously, we have shown that the expansion rate is associated with transcription within the repeat and requires DNA mismatch repair enzymes, MutSβ and the subsequent action of a MutL complex. We established that the necessary MutL complex is the heterodimer of MutL Homologue one (MLH1) with MLH3, which is known as MutLγ. Our studies indicate a pivotal role for MLH3 in GAA•TTC expansion, specifically a single isoform, MLH3 isoform 1 (MLH3iso1). MLH3iso2 does not have an endonuclease domain and does not contribute to expansion in our human cell model. We show that FRDA patient derived cells that we have examined also express MLH3iso1, like our model cells. All of the known MutL complexes require MLH1, while MLH3 is a constituent of only the MutLγ complex. Consequently, when considering therapeutic targets to halt GAA•TTC expansion in FRDA, switching isoforms of MLH3 is much more attractive than targeting other DNA mismatch repair enzyme.

**P014**

## The specific neuronal vulnerability in spinocerebellar ataxia type 1 (SCA1) is not associated with CAG instability between different brain regions

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**Background:** To date there are nine known polyglutamine (polyQ) diseases, which are fatal neurodegenerative disorders, caused by a coding trinucleotide CAG expansion. This CAG expansion is translated into an abnormally elongated glutamine tract in the respective mutant proteins leading to protein aggregation and selective neuronal cell death. Spinocerebellar Ataxia Type 1 (SCA1) is one of the polyQ disorders, affecting 1-3 people per 100,000. It is caused by a CAG expansion in the *ATXN1* gene, which confers a gain of toxic function, resulting in defective transcriptional regulation and RNA metabolism. The length of the CAG expansion inversely correlates with the age at disease onset. Observations have shown that the interruption of the CAG expansion by CAA or histidine (CAT) mutations modulates the effect of the expansion and delays the age at onset. We previously demonstrated (Menon *et al.,* 2013) that in a large cohort of SCA1 patients, 11% had CAT interrupted pathogenic alleles and that the age at onset inversely correlates with the longer uninterrupted CAG stretch. We have the unique opportunity to study DNA extracted from the brain and correlate it to the DNA extracted from the blood of two SCA1 patients. This allows us to study mosaicism in differentially affected tissues.

**Objectives:** This study aims to understand the somatic differences between human blood and brain regions, of the same SCA1 affected individual, variably involved in the neurodegenerative process and their phenotypic effect.

**Method:** DNA was extracted from eight regions of the brain, fragment sized, cloned and sequenced. The fragment sizing was performed using a 3730xl DNA analyser machine. For cloning a 5.4kb plasmid, pcDNA3.1(+) (Invitrogen), was used following procedures described in Menon *et al*., 2013. The same method was used to analyse the DNA previously extracted from the same patients lymphocytes.

**Results:** Firstly, fragment analysis showed that the cerebellum of the SCA1 affected patients presented with less somatic instability compared with other areas of the brain. Secondly, sequence analysis of clones established the sequence of the CAG repeats and found them to be largest in the caudate nucleus and shortest in the cerebellum, with little variation throughout the other regions.

**Conclusion:** These findings argue against a direct association between instability and specific neuronal vulnerability in SCA1, as the cerebellum is the most affected tissue, and overall reveal that CAG instability throughout different tissues correlates poorly with specific neuronal cell degeneration, with no real difference between more affected and less affected regions. However, this could also be due to the fact that the DNA is extracted from some areas of the cerebellum that are more atrophic than other regions of the brain

## P015

### Mitochondrial recessive ataxia syndrome (MIRAS) manifests with obesity and metabolic syndrome

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Mitochondrial recessive ataxia syndrome (MIRAS) is the most common hereditary ataxia in Finland with a 0.9% carrier frequency of the recessive European founder mutation. MIRAS is caused by mutations in *POLG1* encoding for the mitochondrial DNA polymerase (POLG), leading to progressive mtDNA depletion and accumulation of mtDNA deletions. In addition to neurological symptoms (ataxia, dysarthria, neuropathy, epilepsy), MIRAS patients are overweight, which is atypical in mitochondrial disease patients who usually are small and lean. Obesity associated with mtDNA depletion was interesting, as common obesity has also been linked with secondary mtDNA depletion. We characterized here the metabolic features of patients with different types of primary mitochondrial dysfunction: 14 MIRAS patients (overweight), 12 patients with a primary mtDNA point mutation (m.3243A>G; lean), 30 control subjects, and 19 heterozygous carriers of *POLG1* mutation. We utilized magnetic resonance imaging (MRI) and spectroscopy (1H-MRS and 31P-MRS) to determine adipose tissue distribution, intramyocellular lipids, liver fat content and liver metabolites, studied adipose tissue histology, cardiac function, blood lipids, oral glucose tolerance test, and total metabolism by indirect calorimetry. We report that MIRAS leads to abdominal obesity, insulin resistance and high liver fat compatible with non-alcoholic fatty liver disease, not found in patients with primary mtDNA mutation. We conclude that obesity and its associated comorbidities should be considered in the treatment of MIRAS patients. Furthermore, these results demonstrate that mtDNA defects of different types, both leading to respiratory chain deficiency, may result in opposite metabolic consequences – obesity or leanness.

**P016**

## Helper dependent adenoviral vector (HdAV) genetic correction of expanded GAA repeats in Friedreich's ataxia patient specific induced pluripotent stem cells (iPSCs).

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**Background/Hypothesis**: The expanded GAA repeats in the frataxin (FXN) gene form the genetic basis for Friedreich's ataxia (FRDA), and understanding the genetic network related to the repeats would offer tremendous insight on the pathology of FRDA. Gene profiling studies such as microarrays often are confounded by individiual genetic backgrounds, but this bias can be reduced or eliminated by using isogenic cell lines. So far, there have been no reported gene profiling studies performed on FRDA isogenic cell lines. We aim to establish an isogenic FRDA cell line that only differs in repeat length, which allows for more effective and reliable gene profiling studies.

**Methods**: We utilized the HdAV gene correction method, which is an adenoviral based gene targeting approach (see PMID: 21596650). This approach allows for long homology arms (up to 30 kb) that increase gene targeting efficiency, as well as minimal off target effects compared to nuclease based methods (Zinc finger nucleases or TALENs). FRDA patient specific iPSCs were infected with HdAV containing a correction vector for the expanded GAA repeats. iPSC clones were screened for GAA repeat length.

**Results**: The corrected iPSC clones (isongenic to parent clone with unaffected GAA lengths) show restored frataxin mRNA and protein expression levels that are comparable to iPSC clones from unaffected individuals. The histone activation marks infected in FRDA cells such as H3K9 acetylation and repressing marks such as H3K9 di- and tri- methylation all are restored to levels found in iPSCs from uninfected individuals. We have further differentiated the corrected iPSCs into neurons and RNA-seq for gene expression profiling of the isogenic cell lines is currently in progress.

**Conclusions**: We have established an isogenic FRDA iPSC line where the expanded GAA repeats are corrected to six repeats, a repeat length representative of unaffected individuals. We have also differentiated the isogenic iPSCs into neurons. Gene profiling studies on isogenic cell lines will provide insight on the disease pathology and lead to future therapeautic opportunities.

**P017**

## Identification of chemical agents that alter Frataxin protein processing

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Frataxin (FXN) is a mitochondrial protein involved in iron sulfur cluster biosynthesis. FXN is encoded by a nuclear gene to yield a precursor protein (pFXN) that is processed, upon mitochondrial import, into an intermediate form (iFXN) by removal of an N-terminal transit peptide. Within the mitochondria, another transit peptide is cleaved off iFXN to yield the functional mature form, mFXN. Diminished levels of mFXN cause the hereditary disease Friedreich's Ataxia. We surveyed the effect of a set of mechanistically diverse pharmacological agents on FXN processing. Surprisingly, several agents led to distinct changes in the amounts of different forms of FXN, including increasing the relative and absolute abundance of iFXN with no effect on mFXN. Our data suggest that care must be taken when interpreting chemically-induced increases in total FXN levels, to ascertain that they reflect an increase in functional mFXN. Insight into the pathways that control these processing events will be presented.

**P018**

## A family affected by SCA27 caused by interstitial chromosome 13q33.1 deletion

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Our objective is to characterize the phenotype and genotype of a four-generation Swedish kindred affected by a rare spinocerebellar (SCA) type. The phenotype consists of varying degrees of ataxia, abnormal eye movements, cognitive impairment and neuropsychiatric symptoms.

Poly-Q SCA disorders explain up to 50-60 % of all SCA cases. The remaining SCAs are rare and their phenotypes overlapping which makes clinical investigations both time consuming and difficult. Spinocerebellar ataxia type 27 (SCA27) is a very rare SCA form caused by mutations in the FGF14 gene. Haploinsufficiency seems to be a major mechanism of disease in SCA27. Only three families and a sporadic case affected by SCA27 have been reported so far. The first SCA27 family described was a Dutch kindred with 14 affected individuals, their disease was caused by a missense mutation. A recent report (J.A. Coeberg, 2013) described a partial deletion of FGF14 and ITGBL-1 in a SCA27 family.

A comprehensive phenotype work-up that included neurological exam, review of medical charts, imaging studies and psychometric evaluation was carried out on this family. Seven subjects were identified as symptomatic in this kindred, the index case (a 13 y.o. girl) was previously diagnosed with ADHD and postural tremor. Upon closer evaluation she has found to be affected by a mild appendicular ataxia. Despite these symptoms the MRI of her brain was normal. An array CGH (aCGH) was performed first. The subject's mother (34 y.o) is also affected by a similar phenotype and mild ENeG abnormalities. Varying degrees of intellectual disability were found in some of the affected. An interstitial chromosome ~600 kb deletion in 13q33.1 was identified in all the affected subjects. The deleted area contains the entire genes FGF14 and ITGBL-1; the function of the latter gene is unknown. Poly-Q SCAs were ruled out in the index case.

In conclusion, this is the first time a complete deletion of the FGF14 gene is described as the cause of SCA27. Our report supports the role of haploinsufficiency in this disorder. The variable disease expressivity and slow progression in our kindred reminds of the original SCA27 Dutch kindred (J van Swieten et al 2003). However, we also found new phenotype features in this kindred like the presence of cervical dystonia, psychotic symptoms, earlier cerebellar atrophy, spinal cord atrophy and impaired metabolism not only in the cerebellum but also in cortical areas and in the basal ganglia. Finally, aCGH seems to be a useful diagnostic tool in unclear SCA cases.

**P019**

## A new SCA19/SCA22 family with the T377M variant in the KCND3 gene

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SCA19, also allelic with SCA22, is a rare SCA channelopathy caused by mutations of the KCND3 gene. A dominant negative effect is proposed as the mechanism of disease. Only nine SCA19 families of different ethnic backgrounds have been described so far. As in other SCAs, the phenotype is variable and consists usually of mild and slowly progressive ataxia. Myoclonus and cognitive impairment have been described in some cases.

We have found a small family of Swedish origin affected by varying degrees of cerebellar ataxia in which the variant T377M in the KCND3 gene was identified by means of NGS. This variant segregates with the disease and has been described as a putative mutation in a Japanese family (Y Lee et al, 2012). The index case (III:1) in our family is a 43 year old female whose exact age of onset is hard to determine. Since early childhood the subject described clumsiness and inability to perform tandem gait. At the age of 23 years she experienced a clear progression. A MRI of her brain displays mild vermis atrophy and mild white matter abnormalities. Case II:2 mother of the proband is 63 year old and affected by a mild cerebellar ataxia and type 2 diabetes but never considered herself as neurologically affected. She described the presence of head and postural tremor, as well as inability to perform tandem gait since early childhood. The subject describes a non-progressive disease and still ambulatory. Her MRI of the brain displays mild vermis atrophy and widespread white matter abnormalities. Case II:1 is an uncle of the index case who is affected by a severe ataxia that has confined him to a wheel chair. He is also affected by marked dysarthria. His current age is 75 years; age at onset for ataxia was reported to be 21 years. This subject is also affected by a number of complications secondary to his underlying type 2 diabetes (kidney failure). Cognitive impairment is suspected in this case. A sensory polyneuropathy has been found in this case. Marked white matter abnormalities and moderate vermis atrophy were found on a MRI of the brain. The white matter abnormalities were interpreted as microangiopathy. Psychometric evaluation and functional imaging are ongoing.

In conclusion, this is the first time a non-progressive phenotype has been described in SCA19 (case II:2). Also new is the suspected onset during childhood, ethnic background of this family and the presence of polyneuropathy. The latter has to be interpreted with great caution since diabetes is a condition usually associated with damage to periphery nerves. A functional study to ascertain the true pathogenicity of the T377M variant is planned.

**P020**

## Identification, characterization and cloning of a full-length frataxin antisense transcript, FAST-1.

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It has previously been reported that a frataxin antisense transcript, FAST-1, is overexpressed in FRDA patient fibroblasts. However, a lack of detailed information about the FAST-1 gene, including the size, sequence and position of the transcription start and stop sites, has hindered understanding of its potential role in Friedreich ataxia (FRDA). Therefore, we have further investigated FAST-1 in FRDA cells and mouse models.

Firstly, using northern blot hybridization of human fibroblast RNA with two riboprobes, we have identified two distinct bands of approximately 500bp and 9kb, representing potential FAST-1 transcripts. To further characterize FAST-1, we have performed 5'- and 3'-RACE experiments, followed by cloning and sequencing. This analysis has resulted in the identification of a full-length polyadenylated FAST-1 sequence. The 5'- and 3'-ends map to nucleotide positions +164 and -359 of the FXN gene (relative to TSS1 = +1), respectively, giving a total length for this FAST-1 transcript of 523bp. This may correspond to the northern blot band that we determined to be approximately 500bp in size. Interestingly, the start position of this FAST-1 transcript is within a known CTCF binding site.

Subsequently, using a robust qRT-PCR method to quantify FAST-1 expression levels, we have confirmed the original report of increased FAST-1 levels in human FRDA fibroblasts, and we further quantified FAST-1 levels in FRDA mouse model cell lines and tissues. However, no consistently altered patterns of FAST-1 expression were identified in relation to FXN expression. Our full-length FAST-1 clone will be useful for further studies of potential FRDA molecular disease mechanisms.

**P021**

## Analysis of the molecular chaperone-coding gene DNAJB6 as a potential genetic modifier of age at onset in Machado-Joseph disease.

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Machado-Joseph disease/spinocerebellar ataxia type 3 (MJD/SCA3) is an autosomal dominant neurogenetic condition characterized by a CAG trinucleotide repeat expansion in the ATXN3 gene that encodes for an abnormally long polyglutamine (polyQ)-containing protein. Expanded ATXN3 (ATXN3exp) leads to protein aggregation and neuronal degeneration, and symptoms usually start between the third and fifth decades of life. ATXN3exp is inversely correlated to age at onset (AO) of symptoms; however, up to 50% of the variability in AO is not explained by the causal mutation, implying that additional factors, either genetic or environmental, influence AO in MJD/SCA3. Molecular chaperones are compelling modifier candidates, considering their central role in protein quality control. Some chaperones, like DNAJB6, show a strong anti-aggregation potential, and differences in chaperone activity and/or expression levels could be modulating ATXN3exp toxicity and, ultimately, AO. Here we investigated whether genetic variation in DNJB6 could be associated with AO in MJD/SCA3. Five intronic tagSNPs (rs4716704, rs9647660, rs12668448, rs4716707, and rs6459770) from HapMap population data were selected for linkage disequilibrium analysis in 50 healthy individuals and 175 unrelated MJD/SCA3 patients from South Brazil that were previously confirmed by molecular analysis. Patients were further divided into early- (n=19), average- (n=132), or late-onset (n=24) groups (mean±S.E. AO=25.3±1.9, 33.5±1.0, and 48.8±1.5 years, respectively; $p<0.001$), based on multiple regression analysis controlled for ATXN3exp. Allelic, genotypic, and haplotypic frequencies were similar between patients and controls. The same was observed among MJD/SCA3 groups; however, there was a trend ($p=0.068$) for association between rs12668448 genotypes and AO, assuming a recessive model (TT/TC vs. CC; CC frequencies in early-, average-, and late-onset groups equal to 10.5%, 24.2%, and 41.7%, respectively). This difference reached statistical significance when comparing only outlier patients (early- vs. late-onset), assuming a codominant ($p=0.027$; TT vs. TC vs. CC), recessive ($p=0.039$), or overdominant ($p=0.006$; TT/CC vs. TC) genetic model. Preliminary bioinformatics analysis supports a functional role for the genomic region in linkage disequilibrium with rs12668448. ENCODE data show that the 1 kb region comprising rs12668448 is enriched for markers of active regulatory elements, and contains potential DNA binding sites for at least 4 transcription factors which are conserved among primates. Genetic variation in this region may have functional impact on DNAJB6 expression levels, and future studies will be essential to further test the impact of this chaperone on AO in MJD/SCA3 patients.
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**P022**

## Understanding the relationship between normal function and aberrant aggregation: the case of ataxin-3

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Ataxin-3 is responsible for the Machado-Joseph disease, an inherited neurodegenerative disease. This is the most common autosomal dominant spinocerebellar ataxia and a member of the polyglutamine disease family. Biological evidences suggest that ataxin-3 is an ubiquitin-specific cysteine protease, however, despite its medical and biological importance, the physiological function of ataxin-3, about how the protein recognizes ubiquitin targets, as well as the molecular mechanism by which expanded polyglutamine sequences cause selective neurodegeneration remain mostly unknown.
In our group we use an interdisciplinary approach that combines state-of-the-art biophysical techniques such as nuclear magnetic resonance, isothermal titration calorimetry, electron microscopy and high resolution atomic force microscopy with the ambition to capture the complete picture of the molecular interactions between polyubiquitin chains and ataxin-3 and understand how these may affect the aggregation pathway of the protein.
Our approach is based on the assumption that understanding the normal function of proteins is relevant for the design of specific therapeutics to prevent aggregation. This concept is a completely different paradigm from that commonly used which solely focuses on the aggregation states of proteins, and holds the promise of being more effective in drug development.

**P023**

## De *novo KCND3* mutation causes severe Kv4.3 channel dysfunction leading to a unique ataxia phenotype with intellectual disability and epilepsy

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**Background**: Identification of the first de novo mutation in potassium voltage-gated channel, shal-related subfamily, member 3 (KCND3) in a patient with complex early onset cerebellar ataxia in order to expand the genetic and phenotypic spectrum.

**Methods**: Whole exome sequencing in a cerebellar ataxia patient and subsequent immunocytochemistry, immunoblotting and patch clamp assays of the channel were performed.

**Results**: A de novo KCND3 mutation (c.877_885dupCGCGTCTTC; p.Arg293_Phe295dup) was found duplicating the RVF motif and thereby adding an extra positive charge to voltage-gated potassium 4.3 (Kv4.3) in the voltage-sensor domain causing a severe shift of the voltage-dependence gating to more depolarized voltages. The patient displayed a severe phenotype with early onset cerebellar ataxia complicated by intellectual disability, epilepsy, attention deficit hyperactivity disorder, strabismus, oral apraxia and joint hyperlaxity.

**Conclusions**: We identified a de novo KCND3 mutation causing the most marked change in Kv4.3's channel properties reported so far, which correlated with a severe and unique spinocerebellar ataxia (SCA) type 19/22 disease phenotype.

## P024

### The role ferredoxin in Fe-S cluster assembly

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The assembly of Fe-S cluster is an essential pathway whose disruption is associated with severve diseases, most notably Friedreich's Ataxia. De novo synthesis of Fe-S clusters is a highly coordinated process involving a desulphurase enzyme, Fe-S scaffold proteins, ferredoxin, frataxin, and other accessory proteins, whilst the targetting and transfer of clusters to their final protein acceptor requires chaperones and glutaredoxin. To study the roles of these different components, we employ structural and biochemical methods to elucidate how and why the different components interact. In recent work, we have elucidated the structural details of how ferredoxin interacts with the enzyme complex and devised assays to test its function in electron transfer. Our results suggest that ferreodxin is involved in electron transfer in Fe-S clsuter assembly and explain how ferredoxin transfers electrons to the enzyme.

**P025**

## Targeting $(GAA)_n$ repeats and Frataxin protein in Friedreich's ataxia

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Expansion of $(GAA)_n$ repeats in the first intron of the Frataxin (FXN) gene is associated with reduction of mRNA and protein levels and the development of Friedreich's ataxia disease (FRDA). $(GAA)_n$ repeats form non-B-DNA structures, including intramolecular triple-helix H-DNA, which contribute to repeat instability and inhibition of FXN gene expression. Studies have also shown that the FXN protein levels can be regulated by proteasome degradation. Here we aimed to explore two different strategies for obtaining normal levels of FXN protein. First, we mapped H-DNA and higher order structure formation at pathological $(GAA)_n$ repeats in plasmids by using structural- and chemical probing assays. Also, we examined binding of modified oligonucleotides to these repeats to establish their effect on the formation of different DNA structures. Our results demonstrate that sequence-specific binding of modified oligonucleotides at expanded $(GAA)_n$ repeats abolishes H-DNA formation, as indicated by single strand- and triplex-modifying probes. Then we identified the Trim32 protein, with potential E3 ligase activity, as a factor elevating FXN protein levels. Trim32 function is crucial during neuronal differentiation, indicating that this protein has an essential role in the same cell types affected in FRDA patients. We found that in HeLa cells, overexpressing FXN (wt), stabilization of the Frataxin protein is observed when co-expressed with Trim32. Moreover, co-localisation studies using FXN-GFP and Trim32-CFP shows that part of the protein pool co-localizes within mitochondria when overexpressed in HeLa cells. Further results along these lines will be presented.

**P026**

## A novel missense mutation in *ITPR1* causes autosomal dominant non progressive congenital ataxia with mild intellectual disability

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Inositol triphosphate receptors (IP3R) are Ca2+ channels localized at the endoplasmic reticulum and regulate spatiotemporal changes in intracellular Ca2+ signaling, involved in several cellular processes including synaptic transmission, membrane trafficking and gene expression. Type 1 IP3R (ITPR1) is the most abundant isoform in the brain, particularly enriched at cerebellar Purkinje neurons. Mice lacking ITPR1 exhibit ataxia, dystonia, small size cerebellum, impaired cerebellar LTD and abnormal PC dendritic morphology.Deletions of ITPR1 gene have been identified in families with autosomal dominant adult-onset progressive spinocerebellar ataxia (SCA15/16). Missense mutations of *ITPR1* were reported in two SCA15 families and recently in two SCA29 families with non progressive ataxia associated with mild intellectual disability. We describe a two generation kindred of Serbian origin with three affected individuals (mother and two sons) presenting with severe neonatal hypotonia, developmental delay, non progressive ataxia associated with cerebellar atrophy, carrying a heterozygous missense mutation (c.805C>T; p.R269W) in *ITPR1*. The mutation was identified by NGS and validated by Sanger sequencing. The R269 residue is located in the IP3 binding domain of the protein, is highly conserved among species and the mutation was predicted to be deleterious by in silico analysis. Our studies confirm that dysfunction of ITPR1-mediated Ca2+ signaling pathway can be involved in several forms of ataxic phenotypes ranging from congenital non progressive to adult onset ataxia and indicate that ITPR1 gene screening should be performed in families with early-onset ataxia.

**Wednesday 25th March 18:30-20:15**

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**P027**

## Mitochondrial energy imbalance induces oxidative stress and cell death in a Friedreich's ataxia mouse model

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Friedreich's ataxia (FRDA) is a rare inherited neurodegenerative disease. The mutation consists of a GAA repeat expansion within the FXN gene, which down regulates frataxin protein, leading to an abnormal accumulation of iron in the cell. Growing evidence suggests that this may causes changes in mitochondrial function. We now demonstrate that in the YG8R FRDA mouse model mitochondria are deregulated, causing a decrease in mitochondrial membrane potential due to an inhibition of Complex I, and an overactivation of Complex II. This mitochondrial inhibition leads to reactive oxygen species (ROS) generation in the mitochondrial matrix, which results in a massive increase of lipid peroxidation. By counteracting the peroxidation of the lipids we rescue neuronal cell death in YG8R mice. This work describes the physiological properties of the mitochondria in a FRDA mouse model that recapitulates the human phenotype, and show an important target for novel therapeutic strategies.

**P028**

## Characterizing neuronal functionality and mitochondrial activity in Friedreich's ataxia iPS-derived neurons

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Friedreich ataxia (FRDA) is an autosomal recessive disease characterised by neurodegeneration and cardiomyopathy that is caused by an insufficiency of the mitochondrial protein, frataxin. Our previous studies described the generation of FRDA iPS cell lines that retained genetic characteristics of this disease. Here we extend these studies, showing that neural derivatives of FRDA iPS cells are able to differentiate into functional neurons, which don't show altered susceptibility to cell death, and have normal mitochondrial function. Furthermore, FRDA iPS-derived neural progenitors are able to differentiate into functional neurons and integrate in the nervous system when transplanted into the cerebellar regions of host adult rodent brain. One possible explanation for the lack of phenotype in FRDA iPS-derived neurons is that only specific neuronal populations, such as dorsal root ganglia, are sensitive to low Frataxin levels. Another explanation may be that Frataxin protein levels need to be further decreased in FRDA iPS-neurons before overt degenerative cellular mechanisms can be identified. We are currently investigating both these hypotheses by selectively reducing Frataxin expression levels in FRDA iPS-derived sensory neurons. These studies are highly significant for establishing a human cellular model of FRDA neurodegeneration, which can be used for developing FRDA therapies.

**P029**

## Comparative phenotyping of mouse models of Friedreich's ataxia

Melissa Osborne, Catherine Lammert, Laurent Bogdanik, Crystal Davis, Cat Lutz.

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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 (Bradley et.al. 2000). Patients with FRDA exhibit symptoms of incoordination, muscle weakness and sensory loss. In addition, patients also exhibit non-neuronal pathology including cardiomyopathy and approximately 10% of patients will present with diabetes. 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. The Jackson Lab currently has over 15 different mouse models of FRDA under development or for distribution. These models have been genetically standardized and rederived into high barrier facilities for the scientific community. The repository at The Jackson Laboratory (JAX) 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. 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. 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.

**P030**

## Rapamycin reduces oxidative stress by promoting antioxidant defences in a *Drosophila* model of Friedreich's ataxia

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**Background:** Friedreich's ataxia (FRDA) is a form of inherited ataxia with an incidence of 1 in 50000 in Caucasians. The disease is caused by reduction of frataxin synthesis, a mitochondrial protein highly conserved. Numerous studies in patient samples and different model organisms of FRDA support that oxidative stress play a critical role in the pathophysiology of the disease. Currently, there is not an effective treatment for FRDA, therefore, it is crucial to identify new therapeutic targets for this disease.

**Methods:** We used an RNAi-based model of FRDA in *Drosophila* and a genetic screen to identify genes capable of modifying the phenotypes of frataxin depletion in the fruit fly. We treated the model flies with the TORC1 inhibitor rapamycin and studied the effects of this compound on motor performance, survival, ATP production, lipid peroxidation, sensitivity to oxidative stress, aconitase activity and expression of antioxidant defences.

**Results:** We found that genetic reduction on TORC1 signalling improves the impaired motor performance phenotype of FRDA model flies. We also observed that rapamycin treatment restores the motor performance phenotype of frataxin-depleted flies, as well as increases life span and the ATP levels. Furthermore, rapamycin was able to reduce the altered levels of malondialdehyde + 4-hydroxyalkenals of model flies. This protection against oxidative stress is due at least partially to an increase in the transcription of antioxidant genes mediated by *cnc* (*Drosophila* ortholog of *Nrf2*). We observed that autophagy is not critical to protect against oxidative stress in normoxic condition, but it is required in hyperoxia, since rapamycin increases survival and aconitase activity of model flies subjected to high oxidative insult, and that this improvement was abolished by the autophagy inhibitor 3-MA.

**Conclusions:** These results point to TORC1 pathway as a new potential therapeutic target for FRDA, since this pathway modules the energetic and oxidative status of cells.

**P031**

## Screening of phenotypic abnormalities in Friedreich's ataxia-induced pluripotent stem cell-derived cardiomyocytes

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Human induced pluripotent stem cells (iPSC) can be valuable cell models for Friedreich Ataxia (FRDA), as these cells are of patient origin and can be differentiated into cell types of interest for disease modelling, drug screening, and understanding fundamental mechanisms underlying FRDA. We have previously described derivation of two human iPSC lines from individuals with FRDA, and have recently derived a further three iPSC lines, including from FRDA patients with confirmed cardiomyopathy. To date, there are limited reports of a FRDA-related phenotype being identified in the iPSC-derived cell types representative of disease pathology, such as cardiomyocytes. Generating sufficient numbers of cardiomyocytes is a potential limiting factor for further experimentation and characterisation of a FRDA-related phenotype in this disease. We are now utilising methods which exploit small molecule modulators of WNT signalling to differentiate larger and purer populations of cardiomyocytes. These cells are being used in both high density and low density single-cell preparations for assessment of electrophysiological, OXPHOS pathway activities, hypertrophy and small scale assessment of frataxin modulation by a variety of mechanisms. Identification of disease-specific phenotypes in patient iPSC-derived cardiomyocytes is a basic requirement for successful disease modelling, and an essential prerequisite to pre-clinical drug testing. Once established, these models will serve as proof of concept of the value of stem cell derived-cardiomyocytes for the understanding of FRDA pathophysiology and development of new therapeutic interventions.

**P032**

## Generation and characterization of a new neuronal mouse model for Friedreich ataxia

Françoise Piguet[1, 2, 3, 4], Charline de Montigny[1, 2, 3, 4], Nadège Vaucamps[1, 2, 3, 4], Laurence Reutenauer[1, 2, 3, 4], Hélène Puccio[1, 2, 3, 4]

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To date, there is a lack of a good neuronal mouse model for Friedreich ataxia. GAA-based models failed to develop any severe phenotype and the inducible conditional Prp mouse model, with a complete frataxin depletion, develop an ataxic phenotype but with a late onset. Moreover the Prp mice exhibit a severe cerebellar ataxia due to massive granular cell loss. We report here the generation of a new conditional neuronal mouse model for Friedreich ataxia based on the Cre/LoxP technology. We bred mice carrying a conditional allele for the frataxin gene with mice expressing the Cre-recombinase under a sensory neuron specific promoter. Mice were born according to mendelian ratio and did not show any growth abnormalities until 18 weeks of age. As soon as 23 days of age, they exhibit an ataxic phenotype based on bar-test and string test and decrease performances on the rotarod. These phenotypes are progressive, worsening over time. Electrophysiological studies reveal a significant decrease of sensory wave at 4.5 weeks and almost a complete loss at 8 weeks of age. A significant loss of sensory neurons within dorsal root ganglia is observed at 17.5 weeks of age compare to age matched controls at cervical and lumbar levels. Ultrastructural analysis of sciatic and saphenous nerves showed abnormalities as soon as 23 days and progression over time. At 17.5 weeks, many sensory fibers are degenerated and autophagic vacuoles are predominant. Complete histological and biomolecular analyses of this new mouse model are ongoing and will be presented. This new mouse model seems to reproduce many aspect of FRDA and will be useful to study pathophysiology underlying frataxin loss in sensory neurons. Moreover, it will be a useful model to evaluate therapeutical strategy for Friedreich ataxia.

**P033**

## Frataxin knockdown in Drosophila muscles alters mitochondrial homeostasis and degradation

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In Friedreich's ataxia (FRDA), loss of frataxin induces severe mitochondrial dysfunction, affecting predominantly cells with high metabolic rates. Although FRDA is primarily a neurodegenerative disease, frataxin is also crucial in cardiac and skeletal muscle cells. FRDA patients show progressive and symmetrical loss of muscle strength along with defective ATP synthesis, inadequate oxygen utilization and prolonged recovery after exercise.

Drosophila indirect flight muscles (IFM) are thoracic muscles highly enriched in mitochondria and allow easy visualization and monitoring of this organelle. Therefore IFM could be an excellent model tissue to study the effects of frataxin deficiency on mitochondrial function and homeostasis as well as to test the effect and efficiency of possible treatments. For this purpose we have characterized, for the first time, the effects of frataxin knockdown on IFM in Drosophila at histological, biochemical and molecular levels using the specific muscle driver Mef2-GAL4.

To our surprise, severe frataxin deficiency exclusively in muscles was not lethal. However, it induced a dramatic reduction of life span as well as a complete impairment of locomotion ability already in young flies. These results correlate well with the reduced aconitase activity and depleted ATP production in the muscular system of these individuals. The flies also displayed substantial mitochondrial fragmentation. Interestingly, we detected a significant increase of Ref(2)P levels which accumulates in vesicle-like structures. Ref(2)P is the Drosophila homolog of mammalian p62, a cargo protein involved in delivering damaged proteins and organelles to the autophagosome. The initial recruitment of Ref(2)P suggests an activation of mitophagy to degrade damaged organelles. However, the substantial and continuous accumulation of Ref(2)P in our model may indicate an impaired mitophagy in the etiology of FRDA. We are currently determining, by analyzing the exact nature of our Ref(2)P positive vesicles, why mitophagy is inhibited and at which level (endosome, autophagosome or autolysosome) it is interrupted. Additionally, we are modifying the mitochondrial network and promoting cellular mitophagy to study whether counteracting the fragmentation can have a positive influence on our phenotypes.

Altogether, our results demonstrate that Drosophila IFM are an excellent tissue to dissect downstream effects of frataxin depletion. In this work, we describe for the first time an effect of frataxin knockdown on the cellular process of mitophagy.

**P034**

## Cellular and molecular insights into DRPLA from an integrated approach in *Drosophila* and mouse models

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Dentatorubropallidoluysian Atrophy (DRPLA) is an autosomal dominant ataxic syndrome due to expansion of polyQ tract in Atrophin-1 affecting approximately 50 families in the UK. Our laboratory has investigated the cellular mechanism of neurodegeneration in *Drosophila* models, identifying in the block of autophagic flux and in the downregulation of the Fat tumour suppressor gene two critical toxic events (1). Recently, we have expanded our analysis to an existing mouse model for DRPLA (2) and were able to confirm both findings, validating the relevance of our previous work in *Drosophila* and further extending our analysis of neurodegeneration in a model that is anatomically more relevant to the human disease.

Our previous work in *Drosophila* has also revealed a specifically important role of glia in this form of neurodegenerative ataxia, including a striking reduction in lifespan when polyQ Atrophins are expressed in the glia. A sensible hypothesis is that affected glia will act non-autonomously on neurons as in other neurodegenerative pathologies. We have now performed large genetic screens and identified approximately 40 genes and 12 miRNAs that decrease or increase the lifespan of DRPLA flies. Current investigations in this project focus on the role of cell adhesion and signalling transmembrane molecules that are conserved in humans and may be easily targeted given their extracellular role.

*References*

1 I. Nisoli et al., Cell Death Differ 17 (10), 1577 (2010); F. Napoletano et al., The EMBO journal 30 (5), 945 (2011)

2 G. Schilling et al. Neuron 24(1), 275 (1999)

**P035**

## Understanding the pathophysiological and the molecular mechanisms underlying the recessive ataxia ARCA2

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ARCA2, autosomal recessive ataxia type 2, is a cerebellar ataxia characterized by an atrophy of the cerebellum and a mild deficiency in Coenzyme Q10 (CoQ). Moreover a big proportion of the patients show additional neurological signs such as epilepsy and moderate intellectual disability. Different loss of function mutations have been identified in the ADCK3 gene leading to ARCA2. ADCK3 encodes a putative mitochondrial kinase, which is homologous to COQ8 in S.cerevisiae and to UbiB in bacteria, both required for the biosynthesis of CoQ. ADCK3 has been suggested to have a regulatory role in CoQ biosynthesis in mammals. To understand the pathological mechanisms of ARCA2 and study the function of ADCK3, a constitutive Adck3 knock-out (KO) mouse model was generated. Previous results showed that Adck3 KO mice recapitulate many symptoms observed in patients such as the development of a slowly progressive loss of coordination and a mild CoQ deficit, suggesting that they are a good model to study ARCA2. More specifically, Purkinje neurons presented morphological and functional impairment and a mild mitochondrial defect was detected in skeletal muscle. RNAseq analyses of these two tissues implicate ADCK3 in novel cellular processes like vesicular trafficking and lipid metabolism. Here we report the use of cellular models of the affected tissues (cerebellum and muscle) to uncover the molecular signature of ADCK3 loss and CoQ deficit. In particular, we study mitochondrial bioenergetics and lipid metabolism in the muscle cellular model. In parallel, we are setting up cerebellar organotypic cultures to assess the electrochemical properties of Purkinje cells and the possible intracellular trafficking impairment. Altogether, our experiments will shed light on the early molecular events that lead to ARCA2 and may help draw a link between ADCK3 function, CoQ pools and the symptoms we see in patients.

**P036**

## Development and characterization of a new neuronal cellular model of Friedreich ataxia

Françoise Piguet[1, 2, 3, 4], Alain Martelli[1, 2, 3, 4], Charline de Montigny[1, 2, 3, 4], Nadège Vaucamps[1, 2, 3, 4], Pascale Koebel[1, 2, 3, 4], Hélène Puccio[1, 2, 3, 4]

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**Background/Hypothesis:** Friedreich ataxia (FA) is an autosomic recessive sensory ataxia resulting from the decreased expression of frataxin. Although proprioceptive neurons are known to be primarily affected neurons in FA, the molecular pathophysiology implicated in the neuronal dysfunction remains poorly understood. To better characterize and understand the neuropathophysiology in FA, we decided to establish an in vitro model of sensory neurons with complete frataxin deficiency.

**Methods:** Primary cultures of sensory neurons were established from dorsal root ganglia (DRG) of E13.5 mouse embryos carrying a conditional allele for the frataxin gene. Frataxin deletion was achieved through infection of primary cultures with an adeno-associated virus (AAV) expressing the Cre recombinase. Cultures were maintained either as purified neurons or as co-cultures with neurons and Schwann cells.

**Results**: Primary cultures were successfully established and could be maintained in culture up to 42 days after dissection. A time-dependent analysis of frataxin-deficient neurons by electron microscopy showed the progressive appearance of autophagic/mitophagic vacuoles, mitochondrial morphological changes and mitochondrial iron deposits. In parallel, preliminary characterization in co-culture condition indicated that myelin formation was not affected upon depletion of frataxin in neurons.

**Conclusion and perspectives**: We report the first model of sensory neurons in culture with complete deletion of frataxin that display features similar to the ones observed in FA patients. Full molecular and biochemical characterization of the model (e.g. Fe-S-dependent protein levels and activities; expression of stress genes; myelin degeneration) is currently ongoing. In addition, molecular tools are currently being developed to study mitochondrial activity and axonal transport in frataxin-deficient neurons. Collectively, these data should help us to uncover the key molecular pathways involved in the neuropathophysiology.

**P037**

## The role of glia in the pathogenesis of the ataxic syndrome Dentatorubropallidoluysian Atrophy (DRPLA)

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Glial cells are becoming more important in the study of neurodegenerative diseases. It is now clear that they have a major role in neurodegeneration or at least in its progression. We have pioneered the study of Atrophin functions in Drosophila and used this organism to develop models for DRPLA. Mutated Atrophin, containing a polyglutamine stretch, is responsible for DRPLA, an ataxic disease. We discovered that expressing polyQAtrophin specifically in the glia reduces dramatically lifespan. A sensible hypothesis is that affected glia will act non-autonomously on neurons as in other neurodegenerative pathologies.

To understand how mutated glial cells affect neurons, we conducted unbiased genetic screens for mutations in neurons that modify lifespan of Drosophila, expressing polyQ-Atrophin in glia. Downregulation and overexpression of genes have been carried out for a thousand genes as well as overexpression of miRNAs. These screens provided candidate genes that either increase or decrease lifespan.

We will then focus our analysis on the best candidates in order to decipher glia-neuron interactions in a neurodegenerative context. More complex behavioural analysis will be done to further understand the role of these genes before investigating the molecular level.

Our studies will allow us to better understand the impact of mutated glial cells on neurons that finally lead to neuronal dysfunction and death. They will open up new avenues for therapies aimed at targeting glia-neuron interactions during disease progression.

**P038**

## Behavioral and RNAseq analysis of DRG in 3 mouse models of Friedreich's ataxia

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We have carried out an intensive analysis of neurobehavioral deficits in KIKO, YG8 and YG8sR mice, and have also carried out an intensive RNAseq analysis of the complete lumbar tree including DRG in KIKO mice. For neurobehavioral assessments several techniques were used, including: 1) Treadscan analysis of 180 parameters of gait; 2)Locotronic Ladder and level beam for motor coordination; and 3) Von Frey analysis of peripheral sensitivity. YG8 animals had the most obvious motor coordination deficiencies relative to other models and controls. YG8sR mice showed the most decrease in peripheral sensitivity. Intensive RNAseq analysis of KIKO mice produced clear changes in antioxidant genes, Iron-sulfur cluster genes, and mitochondrial genes, consistent with the idea that frataxin-deficiency produces a defect to which DRG responds to by overexpressing protective genes. Some of these changes, including in Myoglobin expression, overlap with transcriptional changes observed in frataxin-deficient human lymphoblasts. Thus, we have identified 1) consistent mouse neurobehavioral deficits that could be used as non-invasive biomarkers of FA progression to test potential therapies in mice, and 2) novel mechanism-related biomarkers in affected tissue, that could potentially be used as biomarkers of progression in human clinical trials.

**P039**

## Frataxin inactivation leads to steroid deficiency in flies

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Friedreich Ataxia (FA), the most common inherited autosomal recessive ataxia in Caucasians, is characterized 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 identify and characterize disrupted pathways caused by frataxin deficiency and to evaluate therapeutic interventions. Ubiquitous inactivation of the fly frataxin ortholog dfh blocks the transition from larval to pupal stages. We investigated the mechanisms by which frataxin deficiency may lead to this developmental phenotype. This study was stimulated by two aims: First, we postulated that it could reveal new physiological functions of Frataxin eventually conserved and affected in the FA disease. Second, this characterization was a prerequisite to determine the relevance of using this phenotype for large-scale drug screening. We show here that frataxin inactivation leads to steroid deficiency and that feeding larvae with the 20-hydroxyecdysone steroid hormone rescues the developmental blockage. In mammals, adrenodoxin is a Fe-S-containing protein essential for the synthesis of various steroid hormones. We show here that Fdxh, the fly homolog of human adrenodoxin, is also involved in steroidogenesis. This provides a potent mechanism by which frataxin, known to be involved in Fe-S cluster biosynthesis, could affect steroidogenesis through reduced adrenodoxin activity. Our data suggest that impaired steroidogenesis is susceptible to occur in FA patients. We are currently testing the effect of frataxin inactivation on steroid hormone synthesis in human cells.

**P040**

## Description of new model of autosomal recessive cerebellar ataxia type 1 (ARCA1) in *Drosophila melanogaster*.

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Autosomal Recessive Cerebellar Ataxia type 1, or ARCA1, is a relatively pure cerebellar ataxia caused by mutations in the SYNE1 gene. Since its first description by Gros-Louis et al. in 2007, 16 SYNE1 mutations in more than 100 patients from Quebec, Japan, France and Brazil have been identified. The numbers of SYNE1 mutations together with the diverse geographical origin of patients suggest that SYNE1 mutations could be a more general cause of pure recessive or sporadic cerebellar ataxia. Deciphering the molecular and cellular contribution of SYNE 1 to ARCA1 is therefore crucial to understand the biology of ARCA1 and propose specific therapeutic targets.

SYNE1 anchors the nuclei to the actin cytoskeleton via its Cterminal KASH domain. All ARCA1 associated SYNE1 mutations identified so far lead to a premature stop of the coding sequence, resulting in SYNE1 KASH domain deletions.

We have shown that Drosophila SYNE1 KASH domain deletion (Msp-300ΔKASH) alters post-synaptic glutamate receptors density (Morel et al., 2014). We present here new results in agreement with SYNE1 involvement in ARCA1 and validating the use of Msp-300ΔKASH flies as a new model for ARCA1.

**P041**

## Cytoskeleton destabilization is involved in neurodegenerative process of dorsal root ganglia from the Friedreich ataxia YG8R mouse.

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Studies in neuronal models of neurodegenerative diseases suggest that the microfilament and microtubule network would be part or early degeneration of neurons exposed to oxidative stress or calcium dyshomeostasis. We have published previously that both process are altered in a neuroblastoma frataxin deficiency model and we have confirmed that these results are reproducible in sensory neurons from dorsal root ganglia (DRG) of the FRDA YG8R mouse model. Thus, we hypothesize that destabilization of cytoskeleton in sensory neurons of DRG may be an early pathogenic event in Friedreich ataxia pathophysiology.

We found in DRG sensory neurons of the FRDA YG8R, neurodegeneration signals like large axonal swellings and beadings. To evaluate the nature of these structures we looked at the expression levels of proteins related with cytoskeleton, axonal transport and mitochondrial marker.

Neurite extension is a complex process that requires precise orchestration of the cytoskeleton, axonal transport and mitochondrial dynamics. We performed morphological and structural analysis in the growth cone and neurites were performed and analyzed by confocal microscopy and immunochemistry. We found a reduction on the area of the growth cones and morphological alterations. It was also observed that YG8R neurons extended shorter neurites than wild type mouse. Additionally, the actin dynamics in growth cones was investigated and we observed an increase of the F-actin expression.

All these dates suggest that a cytoskeleton destabilization would be part of the neuropathological process involved in FRDA pathophysiology.

## P042

### Mitochondrial dysfunction in cellular models of ARSACS

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Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a childhood-onset neurodegenerative disorder caused by mutations in sacsin, a 4579 amino acid modular protein with multiple domains linking it to protein homeostasis[1, 2]. ARSACS patients present with cerebellar ataxia, lower limb spasticity, peripheral neuropathy and pyramidal tract signs. Sacsin is partially localised to the cytosolic face of mitochondria, with loss of sacsin disrupting mitochondrial network morphology and function[3]. Moreover, the N-terminus of sacsin (residues 1-1368) has been reported to interact with the mitochondrial fission factor dynamin-related protein 1 (Drp1)[3]. In this study we perform morphometric analyses of mitochondrial distribution in ARSACS patient cells and identify a phenotype that is consistent with disruption of mitochondrial fission. We also show that mitochondrial association of Drp1 is impaired in sacsin null cells.

To investigate these phenotypes further in a more disease-relevant cell type, we have reprogrammed ARSACS patient fibroblasts to induced pluripotent stem cells (iPSCs) and differentiated them to neurons. Reprogramming was achieved by nucleofecting patient dermal fibroblasts with episomal plasmids expressing the Y4 Yamanaka transcription factors (KLF4, SOX2, OCT3/4, and L-MYC) alongside LIN28 and an shRNA to supress p53 expression[4]. These cells have the advantage over other model systems of being a human model allowing the study of disease-causing mutations in the context of the patient's own particular genetic background.

These cellular models will represent a useful resource for studying ARSACS pathogenesis, including mitochondrial dysfunction, and have potential for screening therapeutics.

*References*

[1]Engert, J.C. et al. Nat Genet. (2000) 24:120-5.

[2]Parfitt, D. et al. Hum Mol Genet. (2009) 18:1556-65.

[3]Girard, M. et al. Proc Natl Acad Sci U S A. (2012) 109:1661-6.

[4]Okita, K. et al. Nat Protoc. (2011) 8:409-12.

**P043**

## Role of the oxidative stress in the genesis of axonopathy in Friedreich's ataxia

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The prominent neurodegenerative feature in Friedreich's Ataxia (FRDA) is a distal length-related axonal degeneration. In order to study whether frataxin gene silencing alters motor neuronal morphology, we have analyzed the morphology of axonal changes in an in vitro neuronal model of FRDA generated by silencing the mouse NSC34 cell line for the frataxin gene. The morphometric analysis performed on this cell line, displaying 40% of residual amounts of frataxin, showed that silenced neurons were almost devoid of neurites, when compared to Mock cell line. Interestingly, this pattern resembled the morphology observed on the NSC34 wild-type after the in vitro treatment with the oxidized form of glutathione, GSSG, and with the specific inhibitor of mitochondrial Complex I, rotenone. Our hypothesis is that the neuronal cytoskeleton is sensitive to the redox imbalance of the couple GSSG/GSH, thus leading to the axonal degeneration and contributing to the "dying back" degeneration in FRDA. Indeed, the cytoskeletal proteins are particularly susceptible to the oxidation and represent the primary target of oxidative damage. Therefore, in order to elucidate the molecular mechanism underlying axonopathy in FRDA, we have studied the in vitro microtubules dynamics by analyzing the polymerization/depolymerization cycles of tubulin in silenced and control cells. We found a consistent increase of the soluble de-polymerized tubulin in frataxin-silenced NSC34 and, noteworthy, this rise was comparable to that observed after GSSG and rotenone treatments. In light of these results we hypothesize that oxidative stress, determined by an increase of intracellular level of GSSG, induces axonal retraction by interfering with microtubules dynamics. These findings also support a role for GSSG as mediator of the alteration of MT dynamics. In addition, the neuronal pre-treatment with the reducing agent N-acetylcysteine appears to slow down the GSSG- and rotenone-driven axonal retraction, further inducing a mild axonal re-growth. In conclusion, we propose a mechanism of axonal degeneration in FRDA, where frataxin deficiency leads to the impairment of mitochondrial CI, GSSG accumulation and de-polymerization of microtubules.

**P044**

## Characterization of the physiological function of Ataxin-1, Ataxin-1L and their native interactor, Capicua, supports a gain-of-function hypothesis for SCA1 pathogenesis.

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Spinocerebellar ataxia type 1 (SCA1) is a devastating neurodegenerative disease caused by an expansion of polyglutamine (polyQ) tract in ATAXIN-1 (ATXN1). SCA1 is one of nine familial disorders in which a polyQ expansion causes the disease protein to misfold, accumulate, and exert toxic functions. We recently discovered that both ATXN1 and its paralog ATAXIN-1-Like (ATXN1L) are strong modifiers of SCA1 phenotypes, pointing to a complex pathological process implicating both gain- and loss-of-function. Thus, understanding the normal molecular and cellular function of ATXN1 is critical to understand SCA1 pathogenesis. Moreover, we noted that ATXN1L serves a likely redundant physiological function to ATXN1 as the double knockout for both is perinatal lethal whereas each individually has little-to-no observable phenotype. In addition, we have previously identified the transcriptional repressor Capicua (CIC) as a native interactor of both ATXN1 and ATXN1L. Interestingly, mice deficient in CIC phenocopy ATXN1/ATXN1L double knockout mice. Nevertheless, due to early lethality, no one has studied the consequence of loss of function of ATXN1/1L or CIC in the adult nervous system. To this end, we generated mice lacking either CIC or both ATXN1 and ATXN1L at an adult stage using a tamoxifen-inducible Cre approach. Surprisingly, we found that these mice do not exhibit any overt phenotype as assessed by gross morphology, survival and behavior. Furthermore, specific loss of ATXN1/1L in the developing hindbrain did not produce overt phenotypes. We are currently performing thorough characterization of each mouse at the behavioral, histological, biochemical and genomic level in efforts to bridge common physiological functions of these proteins. Taken together, these findings support the notion that mutations in the ATXN1 gene result in a gain of toxic function in the cerebellum and brainstem.

MWCR and HCL contributed equally to this work.

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**P045**

## Naïve induced pluripotent stem cells as a new model for Friedreich's ataxia studies (FRDA).

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The ability to derive induced pluripotent stem cells (iPSC) from terminally differentiated human cells creates a novel platform for developing models of disease, regenerative medicine and potential drug discovery. Human embryonic stem cells (ESC) and iPSC share molecular and functional identity with mouse EpiSC (epiblast stem cells) derived from post implantation epiblast (i.e primed state of pluripotency), while mouse ESC demonstrate so called "naïve ground state" of pluripotency and resemble cells in a pre-implantation embryo. Mouse primed cells have limited potential in generating high-grade chimeras in comparison to the naïve cells indicating potentially lesser differentiation capacity of hiPS/hESC. Thus, derivation of human naïve pluripotent stem cells offers practical advantages when comparing differentation potential and improved in vitro growth characteristics such as increased single cell survival, trypsin passages and high proliferation rate.

To derive naïve iPS cells we used non-integration approach to induce pluripotency in fibroblasts obtained from FRDA patient and healthy individual. Reprogramming was conducted in the presence of small molecules: inhibitors of GSK, p38, JNK, ERK/MEK signaling pathways Media was supplemented with bFGF, hLIF and TGF cytokines to create environment optimal for naïve cells derivation. Resulting iPS colonies showed mouse ESC-like dome-shape morphology and were passaged with TryplE with high rate single cell survival.

All cell lines expressed pluripotentcy markers (OCT4, TRA-1-60, SOX2, c-myc, SSEA-4), presented with normal karyotype and after in vitro differentiation demonstrated expression of characteristic markers of all three germ layers (TUJ1- ectoderm; SOX17 – endoderm; SMA – mesoderm). Subcutaneous injections of the naïve iPS resulted in teratoma formation with clearly distinguishable primitive neurotubules, glandular epithelium, smooth muscle cell and immature cartilage. RNAseq data from naïve iPS revealed unique gene expression profile when compared to the primed iPS cells including lower expression of DNMT3A and DNMT3B and up-regulation of DNMT3L and XIST lnRNA.

FRDA naïve-iPS cells expressed higher levels of frataxin than the parental fibroblast line, however significantly lower level when compare to the control naïve iPS cells derived from unaffected fibroblasts. PCR analyses demonstrated that pathological GAA repeats expand significantly with passage number in FRDA patient's naïve cells.

Naïve iPS cells represent an important improvement over primed iPS cells as their high rate of the single cell survival and greater proliferation rate allows for easier and more efficient genome editing of the pathological mutations. Additionally, differentiation potential of the naïve iPS cells may help in generation endodermal and mesodermal cell models of FRDA.

**P046**

## Functional assessment of sensorimotor skills in MCK mutants deficient for frataxin

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Friedreich ataxia (FA) is the most common autosomal recessive disorder of the cerebellum, causing degeneration of spinal sensory neurons and spinocerebellar tracts. The disease is caused by severely reduced levels of frataxin, a mitochondrial protein involved in iron metabolism. We tested an experimental model generated by Dr. Puccio's group by crossing mice homozygous for a conditional allele of the Fxn gene with mice heterozygous for a deleted exon 4 of Fxn carrying a tissue-specific Cre transgene under control of the muscle creatine kinase promoter. Relative to wild-type, the MCK-Cre conditional mutants were impaired on tests of motor coordination comprising horizontal bar, vertical pole, and the rotorod as well as displaying gait anomalies and the hindlimb clasping response. Thus this MCK-Cre model reproduces some key features of muscle dysfunction in patients with Friedreich ataxia and provides an opportunity of ameliorating their symptoms with experimental therapies.

*This work was supported by grants from Ataxie Canada and from Association Française de l'Ataxie de Friedreich.*

**P047**

## Neurological abnormalities in NSE-Cre mutant mouse model of Friedreich ataxia

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The characterization of mouse models of human disease is essential for understanding the underlying pathophysiology and developing new therapeutics. To understand more clearly the pathogenesis of sensorimotor dysfunction in Friedreich ataxia, we used conditional knockout mice in which the frataxin gene had been knocked-out in some tissues during embryogenesis by breeding them with mice expressing the Cre recombinase gene under the NSE promoter. The NSE-Cre mice grew poorly compared to their controls, and demonstrated a severe neurologic phenotype, and a shorter life span. They exhibited extended duration of the hindlimb clasping response, as well as slowed movement time on a suspended bar, severe ataxic gait and kyphosis. The mutants have poor somatosensory perception, breathing difficulties, heart palpitation and autonomic dysfunction as indicated by urogenital failure. Despite the short life span, the NSE-Cre mice are a valid model to evaluate behavioral deficits and could be used to test experimental therapies to improve functional responses.

This work was supported by grants from Ataxie Canada and from Association Française de l'Ataxie de Friedreich.

**P048**

## Understanding disease development in Friedreich's ataxia in a time-resolved way: a new cellular model

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Friedreich's ataxia (FRDA) is a recessive autosomal ataxia caused by reduced levels of frataxin, an essential mitochondrial protein highly conserved from bacteria to primates. The exact role of frataxin and its primary function remain unclear although this information would be very valuable for design a therapeutic approach for FRDA. A main difficulty encountered so far has been that of establishing a clear temporal relationship between the different observations that could allow a distinction between causes and secondary effects and provide a clear link between aging and disease development. To approach this problem, we developed a cellular model in which we can switch off/on the frataxin gene in a time-controlled way partially mimicking what happens in the disease. We exploited the TALEN and CRISPR methodology to engineer a cell line where the presence of an exogenous, inducible FXN gene rescues the cells from the knockout of the two endogenous FXN. This system allows the possibility of testing the progression of disease and is a valuable tool to follow the phenotype with different newly acquired markers.

## P049

### Modelling spinocerebellar ataxia 15 with iPS cell derived neurons

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**Introduction:** Spinocerebellar Ataxia 15 (SCA15/16/29) is the most frequent non-trinucleotide repeat SCA in Central Europe (1). Patients present with a predominantly pure cerebellar ataxia, however, pyramidal, extrapyramidal and cortical features have been reported. MRI-features include primarily vermal cerebellar atrophy as well as mild inferior parietal and temporal cortical volume loss (1, 2). Big heterozygous deletions of variable size in the inositol-3-phosphate-receptor1 are the underlying genetic cause of this disease which is thought to follow a model of haploinsufficiency (3). ITPR1 is highly expressed in the cerebellum as well as cortex and plays an important role in regulation of intracellular calcium & neurotransmitter exocytosis (4). However, the exact underlying pathomechanism leading to cerebellar degeneration is unknown. Here we set out to study the cellular pathology of ITPR1-deletions in a human model of iPSC-derived cortical neurons.

**Methods:** Fibroblasts were collected from 3 SCA15-patients from two different families via skin biopsies. These were then expanded and banked at low passage number. ITPR1-deletion sizes are as follows: patient CT (father) & patient ST (son): deletion-size ~ 310 kb, removing exons 1-3 of SUMF1 and 1-30/40 of ITPR1, patient MD: deletion-size ~ 344408 bp, removing exons 1-3 of SUMF1 and 1-44 of ITPR1. Reprogramming was performed via episomal gene delivery (4). Briefly, cells were nucleofected with episomal DNA containing OCT4, SOX2, KLF4, LIN28, L-MYC and shRNA against p53. From day 28 colonies were picked and expanded as individual iPSC colonies. Two clones per patient were validated and carried further to conduct neural differentiation. Dual SMAD inhibition (5) was used to initiate neural induction, followed by exposure to default neural inductive conditions for 80-100 days to produce mature cortical neurons (6).

**Preliminary Results:** We have successfully reprogrammed fibroblasts from three SCA15-patients. These were confirmed as pluripotent via immunocytochemical staining and qPCR for marker pluripotency genes. The cells presented as karyotypically normal apart from the heterozygous ITPR1-deletion. Successful neurogenesis & data analysis is currently underway.

**Future Work:** We plan to reproduce the current findings and confirm the model of haploinsufficiency of ITPR1-deletions as being the driving force behind the disease. We will use immunocytochemistry, live-imaging and electrophysiology to investigate the interplay between ER, mitochondria and transmitter exocytosis to conclude on the pathomechanism underlying SCA15. Depending on the results, a phenotypic rescue could be investigated via treatment with different calcium-stabilisators (e.g. dantrolene). These findings will help understanding the pathomechanism and ultimately might help in disease management.

*References*:

1. Synofzik et al., J Med Genet, 2011.
2. Novak et al., Mov Dis, 2010.
3. van de Leemput et al., PLOS Genetics, 2007.
4. Kasumu et al., Cerebellum, 2012.
5. Okita et al., Nat Methods, 2011.
6. Chambers et al., Nat Biotechnol, 2009.
7. Shi et al., Nat Protocols, 2012.

**Friday 27th March 12:00-14:00**

**P050**

## Can sensorimotor processing abnormalities explain or contribute to balance impairment in cerebellar disease?

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**Background**: The cerebellum has the potential to participate in balance control as it receives considerable multi-sensory information, known to be important to balance control. Inherited types of cerebellar disease typically feature balance impairment. Balance impairment progresses over time alongside general disease severity but little is known concerning the mechanism through which this develops. Here we investigated spino-cerebellar ataxia type 6 (SCA6) to begin to explore any sensori-motor contribution to the degradation of balance control over time in a relatively well-defined and uncomplicated type of cerebellar disease.

**Objective**: To investigate whether balance impairments in SCA6 are associated with specific sensorimotor processing deficits experiments focused on the cerebellar functions of scaling, coordinate transformation and adaptation of balance responses.

**Methods**: Vestibular, visual and proprioceptive sensory channels were stimulated in isolation using galvanic vestibular stimulation, moving visual scenery and muscle vibration respectively in 16 subjects with spinocerebellar ataxia type 6 (SCA6) and 16 matched healthy controls. Two polarities of each stimulus type typically evoke balance responses of similar form in the forward and backward directions of healthy subjects. Balance responses were measured using whole body motion analysis. Baseline measures of normal body sway were recorded also using motion analysis and disease severity was assessed using the Scale for Assessment and Rating of Ataxia. Baseline measures were compared against response measures to sensory perturbations in order to specifically examine changes which scaled with disease progression.

**Results**: Faster measures of normal body sway confirmed balance impairment at baseline for the SCA6 group ($p=0.009$), which correlated with disease severity ($r=0.705$, $P<0.001$). The SCA6 group exhibited visually-evoked balance responses that were approximately three times larger than normal (backward, $p<0.001$; forward $p=0.005$) and correlated with disease severity ($r=0.543$, $p=0.03$). Vestibular and proprioceptive response magnitudes were not significantly different to healthy controls. Response direction and habituation properties were no different to controls for all three sensory modalities.

**Conclusion**: Sensory perturbations reveal a sensorimotor processing abnormality specific to response scaling to visual stimuli, which could significantly contribute towards balance impairment in cerebellar disease. The absence of decreases in gains of other sensorimotor channels suggests that this is not merely a re-weighting of sensory channels for balance control but rather a potential mechanism for instability. Cerebellar degeneration could disturb the scaling of balance responses evoked by visual motion through disinhibition of extracerebellar visuomotor centres.

**P051**

## Friedreich's ataxia at altitude – Observations during a successful ascent of Mt Kilimanjaro

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**Aims:** A 27 year old male with Friedreich's ataxia (FRDA) and no previous high altitude experience successfully ascended Mt. Kilimanjaro (5895m) over an 8 day period in a wheelchair. High altitude environments can present significant physiological challenges to such individuals, who are thus often denied the opportunity for such travel. Our mentally competent patient was determined to travel regardless, and we sought to support this aspiration in a manner that best mitigated risk. The patient was assessed prior to travel by a multidisciplinary team consisting of neurologists, a neuro-urologist, a cardiologist and a high altitude expert. During the expedition a medic trained in remote mountain medicine accompanied the patient. We describe the pre-expedition assessment, and health observations made during the expedition.

**Methods:** Neuro- and neuro-urological, cardiac (including electro- and echocardiography), pulmonary (including pulmonary function studies) and speech and language assessments were undertaken prior to departure. The subject was accompanied by four male adults aged 22, 23, 29 and 57. All (including the subject) took prophylactic (250mg OD) acetazolamide during ascent. Vital signs and Lake Louise Scores (LLS, used to assess Mountain Sickness) were recorded in the subject and accompanying adults at sea level, 1400m, 2650m, 3479m, 3979m, 4700m and 5895m. The Scale for the Assessment and Rating of Ataxia (SARA) was recorded daily in the subject. The subject's vital signs were compared with the accompanying adults' at sea level, 3479m, 3979m and 4700m using the Student's t-test with Bonferroni correction.

**Results:** Cardiac examination was unremarkable. Pulmonary function studies were inconclusive due to poor technique, although screening for obstructive sleep apnoea was negative (STOP BANG score <3.) Neurological dysfunction, severe prior to departure (wheelchair bound, unable to sit or stand unsupported- total SARA score 28.5), did not deteriorate during or after ascent. LLS were consistently low (<5). Peripheral arterial haemoglobin oxygen saturation ($SaO^2$) fell with ascent [97% at sea level vs 81% at 5895m] as did the accompanying adults (98.25% (CI 95% 97.76-98.74) vs 69.75% (CI 95% 57.84-81.66)). Heart rate increased with ascent in both the subject (94bpm at sea level vs 124bpm at 5895m) and adults (59.75bpm (CI95% 49.81-69.69) vs 119.5bpm (CI 95% 112.78 - 125.72). $SaO^2$ at sea level, 3479m, 3979m and 4700m were similar to the accompanying adults' ($p > 0.05$) whereas the subject's heart rate was consistently higher ($p < 0.0125$ at sea level, 3479m and 3979m – Bonferroni adjustment for multiple comparisons).

**Conclusions:** To our knowledge this is the first reported case of an individual with FRDA travelling to high altitude. During ascent to 5895m we observed no neurological or cardiorespiratory deterioration, nor any other significant medical complication. Such excursions are not without risk, which is hard to quantify in the absence of prior experience. As such it remains unclear whether other patient's with FRDA can safely travel to high altitude.

**P052**

## Rescuing injured or dying Purkinje cells by stem cell fusion

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Degeneration of a distinct region of the brain, the cerebellum, and in particular of Purkinje neurons therein, is common to many neurodegenerative diseases. There are many conditions in which Purkinje cell dysfunction or degeneration occurs, including multiple sclerosis, stroke, metabolic disturbances (such as chronic alcoholism) and cancer. In addition, Purkinje cell loss is a common feature of inherited ataxic conditions, particularly the autosomal dominant spinocerebellar ataxias. Purkinje cell axons represent the sole efferent output of the cerebellum and thus strategies to reduce Purkinje cell injury are crucial to reducing the clinical burden of ataxia.

Of significant relevance to ataxia is the process of mobilising BMSCs into the circulation, subsequent migration and fusion with Purkinje cells in the cerebellum. We have explored Purkinje cell fusion in the brains of patients who had inherited forms of ataxia. Using immunohistochemistry techniques to analyse post-mortem cerebellum tissue, we present evidence of a disease-related increase in Purkinje cell fusion and heterokaryon formation in these patients. Accumulating evidence is therefore raising new questions into the biological significance of cell fusion, with the possibility that it represents an important physiological phenomenon to introduce healthy nuclei or functional genes into aged or degenerating cells that cannot be replaced in adult life.

To investigate the functionality of fused Purkinje cells, using bone marrow chimeric C57BL/6 mice that are stably reconstituted with enhance green fluroescent protein (EGFP)-expressing bone marrow cells; we induced chronic inflammation (through induction of experimental autoimmune encephalomyelitis (EAE) to promote fusion between bone marrow-derived cells and Purkinje cells. In these mice, we show substantial increases in fusion events, which lead to the formation of bi-nucleate heterokaryons that display a typical Purkinje cell morphology and express the GABA-synthesizing enzyme glutamic acid decarboxylase. Identification of Purkinje cells fused with BMSCs, through expression of EGFP, also permitted electrophysiological extracellular recordings to be made of their spontaneous firing, showing that fusion between BMSCs and existing Purkinje cells leads to the formation of electrically active neurons.

In summary, our data provides significant insights into the functionality of fused Purkinje cells. Given this potential solution to repairing Purkinje cells in adult life, harnessing fusion as a potential gene therapy and/or neuroregenerative treatment could be clinically valuable to a vast number of degenerative disorders of the cerebellum.

**P053**

## Identification of proteins and pathways early affected by frataxin deficiency in cardiac myocytes through metabolomics and proteomics approaches

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*Departament de Ciències Mèdiques Bàsiques, Universitat de Lleida, IRB-Lleida, Spain*

We have recently set up a cardiac cellular model of Friedreich Ataxia based on neonatal rat cardiac myocytes and lentivirus-mediated frataxin RNA interference. In this model, frataxin-deficient cardiomyocytes present altered mitochondrial morphology and impaired lipid catabolism. They also show signs of oxidative stress, with the presence of carbonylated proteins and increased sensitivity to the oxidant agent tert-butyl hydroperoxide (Obis et al., Free Radic Biol Med. 2014 73:21-33). In order to identify proteins and pathways early affected by frataxin deficiency in cardiac cells, we performed a metabolomic and a proteomic analysis of these frataxin-deficient cardiomyocytes. Metabolomic analysis was performed by mass spectrometry using an LC-qTOF system. Principal component analysis of these data, revealed the presence of marked changes in the metabolic fingerprint of frataxin-deficient cells. The proteomic analysis was performed by 2D-gel electrophoresis, and allowed us to identify several proteins altered in frataxin-deficient cardiomyocytes. We decided also to explore the presence of protein thiol modifications in frataxin-deficient cardiomyocytes, as several mitochondrial proteins are known to be regulated by cysteine oxidation. With this purpose, the presence of reversible oxidized cysteine residues was investigated using the thiol-reactive fluorescent probe Bodipy-iodoacetamide and 2D-gel electrophoresis. We identified three spots with altered redox status in frataxin deficient cardiomyocytes that were identified by mass spectrometry. The contribution of these identified protein targets on the phenotypes observed in frataxin-deficient cardiomyocytes is currently under investigation.

# Poster Session 5: Drug discovery and emerging therapeutic strategies

**Friday 27th March 12:00 - 14:00**

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**P054**

## HMTase inhibitors as a novel epigenetic-based therapeutic approach for FRDA

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Histone methyltransferases (HMTases) catalyse lysine methylation marks on histone tails and have important roles in a number of cellular processes including DNA repair, replication, transcriptional activation and repression. In Friedreich ataxia (FRDA) cells, such histone modifications have been identified within the *FXN* gene, indicating that the *FXN* gene is subject to heterochromatin silencing. Therefore, inhibition of this repressive histone mark may induce a more open chromatin structure at the *FXN* gene and thus may have a beneficial therapeutic outcome. This study was conducted to assess the therapeutic efficacy of HMTase inhibitors to increase frataxin expression levels and to ameliorate the molecular and biochemical disease effects of FRDA patient fibroblasts and FRDA mouse model cells. We have investigated the use of HMTase inhibitors BIX-10294, GSK126 and UNC0638 at concentrations from 1nM to 10μM in FRDA human and mouse model (Y47R, YG8R and YG8sR) fibroblast cells. 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 a lack of cell toxicity and up to 1.6-fold increases in *FXN* mRNA and frataxin protein levels when using BIX-10294 at concentrations from 1nM to 1μM, consistent in all cell types. In contrast, GSK126 and UNC0638 induced inconsistent changes of *FXN* mRNA and frataxin protein levels in different cell types at non-toxic concentrations, including decreases in *FXN* expression. We conclude that BIX-10294 is a good compound to take forward into FRDA mouse model studies as a potential novel frataxin-increasing therapy. We now aim to investigate the safety and efficacy of BIX-10294 in FRDA mouse models. The outcome of this study may support continued evaluation of BIX-10294 as a novel frataxin-increasing therapy for FRDA and/or provide further information regarding the epigenetics and pathophysiology of FRDA.

**P055**

## Synthetic antisense ATM long non-coding RNA increases in vitro ATM protein levels

Simona Cavalieri[1], Silvia Zucchelli[3], Elisa Pozzi[2], Cecilia Mancini[2], Elisa Giorgio[2], Eleonora Di Gregorio[1], Stefano Gustincich[3], Alfredo Brusco[1, 2]

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Ataxia Telangiectasia is a rare autosomal recessive disorder caused by mutations in the ATM gene. The classical A-T phenotype is characterized by progressive cerebellar ataxia, oculocutaneous telangiectasias, immunodeficiency, hypersensitivity to ionizing radiations, increased risk to developing sinopulmonary infections and predisposition to malignancy. The disease is caused by homozygous or compound heterozygous ATM-null alleles leading to the absence of ATM protein. We studied milder forms of the disease, characterized by slower progression and later age at onset are associated with mutations that leave residual amounts of functional ATM protein (less than 15%). Based on this evidence, and that A-T carriers are healthy individuals with 50% of functional ATM protein, a minimal increase of functional ATM protein up to 30% is likely to restore the physiologic role of ATM.

Here, we present our data on the use of SINEUP strategy to increase ATM protein expression. SINEUPs are a new class of long non coding RNAs (lncRNAs) flanked by repetitive sequences (Short Interspersed nuclear elements, SINEs) that can stabilize and promote the translation of a target mRNA.

We trasfected HEK293 and lymphoblastoid cell lines (LCLs) of healthy donors with Δ5'ATM SINE UP containing the protein-coding target around the ATG (minimal seq around 73bp) of the ATM gene and analyzed mRNA and protein expression by real time PCR and western blotting. In both cases Δ5'ATM SINE UP increased ATM protein synthesis up to 20% compared to scramble, without affecting mRNA levels. Same results were obtained in an A-T heterozygous cell line. Experiments are now in progress on LCLs from milder A-T patients showing a residual amount of functional ATM proteins. Parallel with ATM protein levels we will measure ATM kinase activity with different functional assays.

Our preliminary data indicate that synthetic antisense ATM SINEUP may provide a new therapeutic tool for a group of A-T patients.

**P056**

## Therapeutic discovery for Friedreich ataxia using random shRNA selection

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We screened a 300,000-clone, random shRNA-expressing library and identified shRNA sequences that reverse the decreased growth/survival phenotype of primary Friedreich ataxia (FA) fibroblasts grown in mitochondrial stress media. One of the hit sequences, gFA2, increases frataxin expression ~2 fold, either as a vector-expressed shRNA or as a transfected siRNA. We randomly mutagenized gFA2 to create a gFA2 variant sub-library. We screened this sub-library in primary FA fibroblasts and identified two gFA2 variants, gFA2.8 and gFA2.10, that further increase frataxin expression. Microarray analyses of primary FA fibroblasts expressing another hit shRNA, gFA11, revealed alterations in ~350 mRNAs. Bioinformatic pathway analyses indicated significant changes in mRNAs involved in cytokine secretion; we confirmed significant changes in cytokine secretion induced by gFA11 biochemically. Ingenuity Pathway Analysis revealed that inhibition of a known transcription factor, or treatment of cells with a previously studied chemical compound, induced a statistically similar pattern of gene expression to that induced by gFA11. Inhibition of the transcription factor using a directed siRNA in primary FA fibroblasts, as well as treatment of the cells with the chemical compound, recapitulated the phenotype induced by gFA11, namely reversal of decreased growth/survival in mitochondrial stress media. We are currently planning similar microarray and bioinformatic analyses of the optimized versions of gFA2. Combined with microarray analyses and bioinformatic pattern-matching, our random, shRNA library screens potentially yield, 1) small-RNA therapeutic candidates, 2) conventional chemical-compound therapeutic candidates, 3) drug-target candidates, and 4) elucidation of disease mechanisms, which may inform additional therapeutic initiatives.

**P057**

## Tauroursodeoxycholic acid reduces neuroinflammation and improves motor symptoms in a transgenic mouse model of Machado-Joseph disease

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Machado-Joseph disease (MJD), also known as Spinocerebellar Ataxia type 3 (SCA3), is an autosomal dominant neurodegenerative disorder for which no effective treatment is currently available. We have generated a new transgenic mouse model expressing human ataxin-3 with an expanded CAG tract ubiquitously and at near-endogenous levels. CMVMJD135 mice develop a severe and progressive neurologic phenotype, with intranuclear inclusions in neurons and brain pathology consistent with the human disease.

Tauroursodeoxycholic acid (TUDCA) is a bile acid (BA) with neuroprotective action, through its anti-amyloidogenic and chemical chaperone activities and its ability to modulate apoptotic pathways. This BA is orally bioavailable, BBB permeable, and has a very low toxicity profile. In addition, TUDCA has been approved by FDA for use in humans to treat liver disorders. TUDCA has been shown to be beneficial in several models of different neurodegenerative diseases. In this study, the chronic treatment of CMVMJD135 mice and wild-type littermates with 0.4% TUDCA supplementation in the food was administered pre-symptomatically from 5 to 34 weeks of age. The behavioral analysis was performed every two weeks using a battery of neurological tests. Our results show that food supplementation with TUDCA (i) delayed the onset and improving the motor phenotype observed in the motor swimming test; (ii) partially improved the muscular strength deficits observed in the CMVMJD135 mice; (iii) improved some neurological parameters, such as limb clasping and tremors, but not exploratory movement deficit, gait quality and the decrease in body weight gain and (iv) delayed and improved the foot dragging phenotype. We also observed that TUDCA treatment was able to reduce the steady-state levels of mutant ataxin-3 and to normalize the levels of TNF-a in the brain of CMVMJD135 mice. This suggests that TUDCA is acting through its anti-inflammatory properties. In the future, we aim to assess the effect of TUDCA on: (i) ataxin-3 aggregation; (ii) brain pathology; (iii) ER stress, autophagy and other proteostasis branches.

**P058**

## Developing novel gene and cell therapies for Friedreich ataxia

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Debilitating neurological disorders such as Friedreich ataxia (FRDA) have limited treatment options in the clinic. The primary clinical features of FRDA include progressive ataxia and shortened life span, with complications of cardiomyopathy being the major cause of death. FRDA is most commonly caused by homozygous GAA trinucleotide repeat expansions in the first intron of the frataxin gene (FXN), which leads to reduced levels of frataxin; a mitochondrial protein important for iron metabolism. The GAA expansion in FRDA does not alter the coding sequence of FXN. It results in reduced production of structurally normal frataxin and hence any increase in protein level is expected to be therapeutically beneficial. Current treatments aim to improve quality of life, but none can cure or slow the progression of neurodegeneration inherent to this disease. Novel therapeutic applications like gene therapy combine two cutting-edge technologies in clinical research: gene correction and cell therapy. Each has significant potential in its application to effectively treat neurological disease. We are developing a series of lentiviral vectors that express FXN to increase frataxin expression in cells derived from individuals with FRDA. Our goal is to deliver the most promising vector via autologous bone marrow transplant to correct FRDA. We therefore examined the potential of transplant with wild-type donor bone marrow (BM) to correct frataxin deficiency in an FRDA mouse model. Hematopoietic reconstitution in FRDA mouse recipients with GFP-positive donor BM demonstrated successful engraftment following transplant. Corrected recipients demonstrated low-level chimerism in other tissues post-transplant, with GFP-positive cells detected in the dorsal root ganglia and the spinal cord. We are currently performing expression and behavioural analyses to determine if there is increased frataxin expression and any improvement in the locomotor phenotype in corrected FRDA mice. This study highlights the potential of bone marrow transplant as an alternate treatment approach for FRDA.

**P059**

## Exploring the therapeutic potential of ataxin-3 protein modification in spinocerebellar ataxia type 3 patient-derived neurons and transgenic mice

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Spinocerebellar ataxia type 3 (SCA3) is caused by a polyglutamine expansion in the ataxin-3 protein, resulting in gain-of-toxic function and aggregation of the mutant protein. There have been several studies describing gene silencing approaches to lower ataxin-3 protein levels as a treatment for SCA3. However, ataxin-3 plays an important role in protein deubiquitination and degradation and complete downregulation is likely to have detrimental effects. Therefore, a treatment strategy that would reduce toxicity of the ataxin-3 protein while retaining its cellular function would be a major improvement.

We have recently shown the removal of the toxic polyglutamine repeat from the ataxin-3 protein through antisense oligonucleotide-mediated exon skipping *in vitro* in patient-derived fibroblast and *in vivo* in control mice. With two antisense oligonucleotides we were able to skip two exons from ataxin-3, which resulted in a modified ataxin-3 protein lacking the toxic polyglutamine repeat. Our approach is unique as it removes the toxic part of the ataxin-3 protein while preserving as much as possible normal ataxin-3 protein function. While these results are very promising, for future clinical application and EMA/FDA approval it would be preferable to use only one antisense oligonucleotide. Currently we are testing the therapeutic potential of a novel approach where only the exon of ataxin-3 that contains the CAG repeat is skipped.

The traditional model systems to study neurodegenerative disorders are typically of non-neuronal or transgenic origin. Here we make use of SCA3 patient-specific neurons derived from pluripotent stem cells. With SCA3 patient-derived neuronal cells it was shown for the first time that neuronal excitation results in formation of SDS-insoluble ataxin-3 aggregates. This disease-relevant phenotype in a neuronal cell model for SCA3 provides the unique possibility to show a phenotypical improvement after antisense oligonucleotide-mediated ataxin-3 protein modification. The first results obtained from SCA3 patient-derived neuronal cells showed the feasibility of antisense oligonucleotide-mediated formation of modified ataxin-3 protein lacking the polyglutamine repeat.

The use of a SCA3-relevant animal model will be crucial to further assess efficacy of the current therapeutic approach. To study exon skipping *in vivo*, we make use of an animal model with the complete genomic human *ATXN3* gene. The MJD84.2 transgenic mouse harbors a YAC transgene that express a human *ATXN3* gene modified with an expanded 84 CAG repeat motif. With age, the mice show a clear motor phenotype as well as marked neuronal degeneration and ataxin-3 aggregation. Repeated *in vivo* injections of the antisense oligonucleotide were performed in the brain ventricle of MJD84.2 mice. Widespread antisense oligonucleotide distribution and ataxin-3 exon skipping was found throughout the brain. Next, we are planning to study behavioral improvements after antisense oligonucleotide-mediated ataxin-3 protein modification.

**P060**

## Caffeine alleviates progressive motor deficits in Machado-Joseph disease transgenic mice

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Machado-Joseph disease (MJD) is a dominantly inherited neurodegenerative disorder associated with an expanded polyglutamine tract within ataxin-3 for which there is currently no available therapy. Since caffeine, a non-selective adenosine receptor antagonist, reduced the neuropathological modifications triggered by lentiviral-mediated over-expression of mutant ataxin-3 in the mouse striatum (Ann Neurol 2013; 73:655-666); we now investigated its ability to also alleviate behavioural deficits in a genetic mouse model of MJD displaying severe ataxia associated with the expression of mutant ataxin-3 in the cerebellum (EMBO Rep. 2008 9:393-399). For this purpose, MJD transgenic mice were given caffeine (1 g/L, applied through the drinking water) and were behaviourally tested using a panel of locomotor paradigms, namely rotarod, beam balance and walking, and pole tests.

Our results showed that chronic administration of caffeine prevented progressive loss of general and fine-tuned motor functions, balance and grip strength, in parallel with cerebellar morphology preservation, which were presumably operated by the adenosinergic neuromodulation system in the cerebellum. Finally, these findings provide the first in vivo demonstration that caffeine intake alleviates motor disabilities in a severely impaired animal model of MJD.

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**P061**

## Purkinje cell replacement in adult SCA2 and WT cerebella: pharmacological and genetic PI3K pathway inhibition

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Spinocerebellar ataxia (SCA) is a genetic neurodegenerative disorder. Many forms of SCA are characterized by a severe Purkinje cell (PCs) loss. The availability of strategies aimed at replacing PCs lost to the disease would eventually facilitate the identification of a cure for SCAs. Several research teams have approached the treatment of SCAs using transplantation of various types of progenitor cells and have shown that while PC progenitors properly integrate when grafted to an immature host, if transplanted into the adult cerebellum they fail to functionally integrate in the host cytoarchitecture and establish cortico-nuclear connections. Studies of external granular layer (EGL) ablation or temporary neutralization of Reelin signaling have demonstrated that Reelin secreted by granule cells is responsible for the observed barrier effect to cell replacement. Taken together, these results indicate that the proper integration of grafted PCs could potentially be achieved by manipulating the host environment, or the responsiveness of grafted cells to Reelin signals. Previous studies have shown that Reelin regulates PCs migration through phosphorylation of the intracellular adaptor Disabled-1, which, in turn, activates phosphatidyl-inositol 3 kinase (PI3K).

In order to interfere with the inhibitory cues of Reelin through PI3K pathway we used a double approach: 1) pharmacological study. We tested both *in vitro* and *in vivo* an inhibitor of PI3K. In organotypic co-cultures of E12 L7-GFP+ cerebellar primordia with wt postnatal day 9 cerebellar slices, treated donor cells left the source tissue, invaded the host EGL, and settled in the molecular layer (ML). Vehicle-treated donor cells did not cross over to the host slice. In heterochronic transplantation, treated L7-GFP+ PC progenitors, grafted to the adult cerebellum of SCA2 and wt mice, migrated along the host granular/molecular layer interface and integrated with their soma within the host PC layer, replacing missing PCs. Integrated donor PCs grew dendrites into the host ML and projected axons through the host internal granule layer (IGL). Conversely, vehicle-treated donor cells grafted to SCA2 or wt cerebella, either settled ectopically in the host white matter, or migrated along unusual routes, reaching the ML or IGL, invariably failing to integrate into the host PC layer. 2) genetic study. We produced a lentivirally encoded Dab-1 shRNA. Infected donor PC progenitors, grafted to the adult wt cerebellum, migrated radially along the Bergmann fibers of the host molecular layer towards the PC layer.

To fully analyze the effect of the PI3K inhibitor and the shRNA Dab-1 lentivirus, we need to test the formation of cortical-nuclear connections and to test the functionality of grafted donor cells with electrophysiological measurements.

Our results could represent a valid contribution to cell therapy approaches aimed at replacing diseased PCs in SCA patients.

**P062**

## Incretin analogs as new therapeutic agents for Friedreich's ataxia

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**Background and aims:** Friedreich's ataxia (FRDA) is associated with a high prevalence of glucose intolerance and diabetes. We have previously demonstrated that dysfunction and death of insulin-producing pancreatic β-cells is central for diabetes development in FRDA. The molecular mechanisms underlying this process are still unknown. Incretin analogs are drugs currently used for treatment of type 2 diabetes. They induce cAMP formation and improve β-cell function and survival. We have previously shown that incretin analogs are protective in frataxin-deficient β-cells, suggesting that they may have therapeutic potential in FRDA. Our aim in the present study was to elucidate the molecular mechanism(s) of frataxin deficiency-induced apoptosis in FRDA, and clarify how is this modulated by incretin analogs.

**Materials and Methods:** Frataxin was silenced in β-cells using RNA interference. Inducible pluripotent stem cells from two FRDA patients and one control, two clones of each, were differentiated into neurons. Mitochondrial $H_2O_2$ production and mitochondrial glutathione redox state were monitored using HyPerMito and mt-roGFP1 probes, respectively. MnTMPyP and Tiron were used as ROS scavengers. RNA interference was used to silence BAD, DP5, Puma or Bim (≥ 50% knockdown) in frataxin-deficient β-cells. The cAMP inducer forskolin was used at 20 μM, and the incretin analog exendin-4 at 50 and 500 nM in β-cells and neurons, respectively. Protein and gene expression was analyzed by Western blot and real-time PCR. Apoptosis was examined by Hoechst/propidium iodide staining, caspase-9 activation and cytochrome-c release.

**Results:** Frataxin silencing in β-cells increased mitochondrial $H_2O_2$ production and enhanced glutathione oxidation (n=3-8, $p<0.05$). Frataxin deficiency activated the intrinsic pathway of apoptosis in β-cells and FRDA neurons, as indicated by mitochondrial cytochrome-c release and caspase-9 cleavage. ROS scavenging reduced apoptosis in frataxin-silenced β-cells (n=3-4, $p<0.05$). Frataxin silencing in β-cells induced the Bcl-2 pro-apoptotic proteins DP5, Puma, and Bim (n=9-14, $p<0.05$), and reduced BAD phosphorylation. Bim was also induced in FRDA neurons (n=5-7, $p<0.05$). Silencing of DP5, BAD and Bim, but not Puma, reduced frataxin deficiency-induced apoptosis in β-cells (n=4, $p<0.05$). Forskolin treatment normalized mitochondrial oxidative status and prevented activation of the intrinsic pathway of apoptosis in frataxin-deficient β-cells and neurons. Importantly, forskolin and exendin-4 induced frataxin protein expression in β-cells and neurons, with exendin-4 increasing frataxin expression by 1.6±0.2 fold in FRDA neurons (n=12 $p<0.05$).

**Conclusions:** Mitochondrial oxidative stress-mediated activation of the intrinsic pathway of apoptosis is a common mechanism of cell death in β-cells and neurons in FRDA. cAMP induction effectively prevents this process. Forskolin and exendin-4 also induce frataxin protein expression in β-cells and neurons from FRDA patients, which may represent a second cytoprotective mechanism. Based on these exciting observations, we propose that incretin analogs should be tested as a novel strategy to prevent or delay both neurodegeneration and diabetes in FRDA.

**P063**

## Serotonergic signaling suppresses Machado-Joseph disease pathogenesis

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Machado-Joseph disease (MJD) is a neurodegenerative disorder caused by the expansion of a polyglutamine (polyQ) tract within the C-terminal of the ataxin-3 (ATXN3) protein. Mutant ATXN3 acquires the ability to self-associate and enter an aggregation process, which is associated with several pathophysiological consequences for neurons. The lack of therapeutic strategies that effectively prevent neurodegeneration in MJD patients prompted us to search for compounds that modulate mutant ATXN3-related pathogenesis. We conducted a secreening of ~1200 mainly FDA-approved out-of-patent compounds for their ability to ameliorate mutant ATXN3-mediated neuronal dysfunction. Our hits clustered into seven major therapeutic groups: neurotransmission; anti-infective; cardiovascular; anti-inflamatory; hormone substituent; analgesic; and anti-asthmatic. All compounds rescued or ameliorated motility defects, with a subset of the small molecules also decreasing mutant ataxin-3 aggregation, without major changes in protein levels. Surprisingly was that modulation of the serotonergic pathway was effective on restoration of motility. By combining compound treatment with genetic tools, we demonstrated that modulation of serotonergic signaling by selective serotonin re-uptake inhibitors (SSRIs) suppressed mutant ATXN3 aggregation and neurotoxicity in C. elegans. Specifically, treatment with citalopram rescued mutant ataxin-3-mediated neuronal dysfunction and ameliorated aggregation in vivo. MOD-5, the C. elegans ortholog of the serotonin transporter in vertebrates and cellular target of citalopram, was confirmed as a strong genetic modifier of MJD and was shown to be necessary for therapeutic efficacy, as were serotonin receptors. These results suggest serotonergic signaling to be a promising therapeutic target for the delay of MJD progression. Furthermore our strategy revealed to be a strong approach by which safe and highly effective bioactive small molecules can benefit complex diseases of protein conformation.

**P064**

## Stem cell-mediated neuroprotection in a mouse model of Friedreich´s ataxia

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In this work we have analyzed both in vitro and in vivo the effect bone marrow-derived mesenchymal stem cells (MSC) exerts on the dorsal root ganglia (DRG) of a Friedreich´s ataxia (FA) mouse model, the Fxntm1Mkn/Tg(FXN)YG8Pook transgenic mouse. In our first study using DRG cultures, we demonstrated that MSC release certain trophic factors that stimulate neuroprotective mechanisms and increase the survival of both neurons and satellite/Schwann cells when submitted to oxidative stress. Besides increased cell survival, an upregulation of oxidative stress-related genes as well as frataxin at both the genetic and protein level were observed. This effect was observed using MSC isolated from wildtype mice as well as those from FA transgenic mice, indicating that autologous stem cell transplantation may be feasible.

In our in vivo study, intrathecal injections of mesenchymal stem cells were performed on the lumbar region of the spinal cords of FA mutant mice. The mice were submitted to behavior tests for several months before the transplantation procedure, in order to analyze their motor skills. These tests included rotarod (which analyzes coordination and balance) and treadmill (to study the maximum speed attained). After this period, the mice were submitted to the surgical intervention, using mesenchymal stem cells from either wildtype or YG8 mice. Also, wildtype mice of the same age and sex were used as a control of the disease. All groups of mice were submitted to the behavior tests for an additional 5 months. As a result, the scores of the behavior tests in the MSC-treated mice were significantly better than the non-treated mice. This was due to the presence of the MSC in the dorsal root ganglia, which released various trophic factors that are known to increase cell survival. Various techniques were used to analyze the dorsal root ganglia, including immunohistochemistry, western blot, quantitative PCR, and electron microscopy. These studies demonstrated that the DRGs of the MSC-treated mice presented increased cell survival markers and subsequently decreased degeneration, increased levels of several antioxidant factors, as well as more frataxin expression.

Overall, the results observed demonstrate that MSC transplantation protects the neurons from the dorsal root ganglia from degeneration in an FA mouse model, which results in the mice presenting better motor skills compared to the non-treated mice. The fact that MSC isolated from FA mice presented similar results to those observed from wildtype mice indicate that autologous stem cell transplantation in patients with FA may be feasible.

**P065**

## A small neurotrophic factor receptor agonist prevents neurodegeneration and increases frataxin expression in experimental models of Friedreich's ataxia

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Neurotrophic factors contribute to the maintenance of neuronal cell survival, connectivity and plasticity. Interestingly, numerous studies have demonstrated the potential of neurotrophic factors for the treatment of a variety of neurological diseases, particularly on some neurodevelopmental and neurodegenerative diseases. However the therapeutic application of most neurotrophic factors has been very seriously limited because of their poor pharmacokinetic properties. In order to address these limitations, various strategies are now being pursued. These include both the localized expression of neurotrophic factors via viral and cell-based delivery systems and the development of small molecules targeting neurotrophic factor receptors with better pharmacological properties. One of these chemicals, 7,8-dydroxyflavone (7,8-DHF), has been reported to activate TrkB brain-derived neurotrophic factor (BDNF) receptors and protect neurons from degeneration in a variety of experimental models.

Thus we have explored the possibility that 7,8-DHF may be neuroprotective in experimental models of Friedreich's ataxia (FA), a neurogenetic disease caused by a deficit in the expression of frataxin.

Our results indicate that 7,8-DHF is able to protect primary cultures of mouse neurons against apoptotic cell death caused by the knockdown of frataxin gene expression. Moreover, 7,8-DHF treatment also decreases the level of apoptotic markers in primary neuronal cultures from YG8 mice (which are homozygous for the knocked-out mouse frataxin gene and hemizygous for the human frataxin transgene bearing the mutation which is responsible for FA). Interestingly, 7,8-DHF treatment increases the level of frataxin in primary neuronal cultures from both wild-type and YG8 mice. Our preliminary results indicate that this is due at least in part to up-regulation of transcription.

Furthermore, chronic 7,8-DHF treatment of olfactory mucosa stem cells, obtained from biopsies from FA patients, leads to an increase in frataxin expression which is paralleled by an enhancement in aconitase enzyme activity.

In view of these data we suggest that small molecule neurotrophic factor receptor agonists such as 7,8-DHF may be useful to slow down neurodegeneration in Friedreich`s ataxia. Further experiments in vivo in mouse models are required to support this possibility.

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**P066**

## Herpesvirus FXN vectors for non-invasive, physiologically regulated gene therapy of Friedreich's ataxia

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Safe, non-invasive transgene delivery to spinal cord tissues has been demonstrated using HSV-1 vectors in a clinical trials to treat intractable pain (David Fink, Michigan). In these human studies successful transduction has been achieved of dorsal root ganglion neurons, also principal targets of gene therapy for Friedreich´s ataxia. Following on from these encouraging results, and our previous work demonstrating longterm expression driven by the FXN genomic locus, we generated a novel recombinant HSV-1 vector deleted for more viral genes than the vectors used in Michigan, thus offering a much larger transgene capacity and an improved toxicity profile. We have now inserted into the new HSV-1 vector, a trimmed (23 kb) version of the FXN gene consisting of [5 kb promoter-exon1-intron1-exons2-5 cDNA] which we designed to maintain important elements of physiological FXN regulation for longterm gene therapy in FRDA patients. We present results on the characterization of this novel vector with the aim of translation to the clinic.

**P067**

## Investigation of diazoxide as a novel frataxin-increasing therapy for Friedreich ataxia

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Looking for molecules involved in the modulation of frataxin expression, we found a slight inhibitor effect of rapamycin, the well-known inhibitor of mTOR kinases. Therefore, we decide to study the expression of frataxin mRNA and protein in the presence of mTOR modulators, using human embryonic kidney cells (HEK293) and lymphoblastoid cell lines from Friedreich ataxia (FRDA) patients. Particularly, we tested the positive regulators of this pathway as glucose, leucine and diazoxide. Diazoxide, a drug commonly used as vasodilator in the treatment of acute hypertension, is able to activate mTOR pathway in adult rodent islets in the presence of chronic exposure to glucose. We found that 4 days of treatment improved the amount of frataxin protein in both HEK293 and immortalized lymphocytes from FRDA patients. Moreover, we studied the therapeutic efficacy of diazoxide, to increase frataxin expression levels and to ameliorate the functional and biochemical disease effects of two FRDA mouse models: the YG8sR mice developed in the Pook lab and the KIKOΔneo mice developed in the Pandolfo lab and modified by The Jackson Laboratory. Prolonged oral administration of 3mpk/d diazoxide in two FRDA mouse models was found to be safe, but produced variable effects concerning efficacy. Thus, both FRDA mice showed improved beam walk coordination abilities, while only KIKO mice showed improved rotarod performance and only YG8sR mice showed significantly increased frataxin expression, improved aconitase activity and decreased protein oxidation in cerebellum and/or brain mitochondrial tissue extracts. Further studies are required before diazoxide should be considered for FRDA clinical trials.

**P068**

## Excision of expanded GAA repeats alleviates the molecular phenotype of Friedreich's ataxia

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Friedreich's ataxia (FRDA) is an autosomal recessive neurological disease caused by large homozygous expansions of the GAA repeats in the intron 1 of the frataxin (FXN) gene. Expansion results in epigenetic silencing of the FXN locus and significant decrease of frataxin expression. We report that human FRDA lymphoblasts and patient fibroblasts can be corrected by the zinc finger nuclease mediated excision of the expanded GAA repeats and that the correction persists during reprogramming to the induced pluripotent stem cells (iPSCs) and further during differentiation into neurons. Editing of a single expanded GAA allele created heterozygous, FRDA-carrier like cell lines and significantly increased frataxin expression. Further, ZFN-mediated editing normalized expression of FRDA biomarkers in corrected patient lymphoblasts and reversed disease phenotypes, such as aconitase activity and intracellular ATP levels, in iPSC-derived neuronal cells. The ability to obtain patient-specific, genetically corrected iPSCs and neuronal cells from FRDA patients provides not only the disease-relevant models in identical genetic backgrounds but also is an essential step in development cell replacement therapy. Genome editing developed herein may have a broader significance towards more than 30 other diseases caused by expansion of repeat sequences.

**P069**

## CAT-4001, a novel NRF2 activator and NF- κB inhibitor, for the treatment of Friedreich's ataxia

Andrew Nichols, Michael Jirousek, Michael Zimmer, Dominic Picarella, Pradeep Bista

*Catabasis, Cambridge, US*

In Friedreich's ataxia (FA) the deficiency of frataxin expression leads to oxidative stress that may play a significant role in disease neuropathology. Impaired nuclear translocation of Nrf2, a transcription factor that regulates cellular responses to oxidative stress, may be the causative factor for the oxidative neuronal damage in FA. In addition, neuroinflammation may play a role in the pathology of FA. Thus, activation of Nrf2 to drive antioxidant pathways and inhibition of NF-κB to reduce inflammation may represent an approach to the treatment of FA.

CAT-4001 is a novel small molecule of monomethyl fumarate (MMF) conjugated to the omega-3 fatty acid, docosahexaenoic acid (DHA), through a linker designed to be cleaved by a specific intracellular enzyme to enable simultaneous intracellular release and pharmacological activity of its active components. MMF activates Nrf2 and DHA inhibits NF-kB. Thus, CAT-4001 was designed to allow the simultaneous intracellular delivery of MMF and DHA to activate Nrf2 and inhibit NF-κB in the same cell at the same time.

CAT-4001 was taken into cells and trafficked to the endoplasmic reticulum where it was hydrolyzed by fatty acid amide hydrolase to release MMF and DHA. As a result, CAT-4001 inhibited the activation of NF-κB, subsequent p65 DNA binding and release of inflammatory mediators in THP-1 and human peripheral blood mononuclear cells (PBMC) to a significantly greater degree than either DHA or MMF either alone or in combination at the equivalent concentrations. Similarly, CAT-4001 activated Nrf2 significantly more potently than MMF or DHA, either alone or in combination, as assessed by HMOX1 mRNA induction in THP-1 cells. CAT-4001 inhibited LPS-stimulated increases in plasma TNFa by up to 70% after a single oral dose in rats, but equimolar equivalents of DHA and MMF given in combination had no effect. Likewise, a single oral dose of CAT-4001 given to mice activated Nrf2 as determined by significantly increased HMOX1 mRNA expression in the kidney, whereas an equimolar dose of the precursor to MMF, dimethyl fumarate, had no effect. In dogs, a single oral dose of CAT-4001 simultaneously increased basal HMOX1 mRNA expression in PBMC and inhibited LPS-stimulated increases in PBMC TNFa, IL6, CCL2 and IL1B mRNA expression. In lymphoblastoid cell lines from FA patients basal HMOX1 expression was lower than in their unaffected parent, confirming lower basal activation of Nrf2 in FA. However, CAT-4001 increased HMOX1 expression equally well in FA and normal cells. In addition, CAT-4001 increased expression of the Nrf2 target genes, HMOX1, NQO1 and SOD2, equally well in immortalized fibroblasts from FA patients and unaffected individuals.

In conclusion, CAT-4001 simultaneously activates Nrf2 and inhibits NF-κB, and may represent an effective approach to activation of antioxidant pathways and inhibition of inflammation for the treatment of FA.

**P070**

## Exendin-4 improves β-cell function and glucose tolerance in KIKO mice

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**Background and aims**: Friedreich's ataxia (FRDA) patients have increased body fat content and are insulin resistant. They develop diabetes when their insulin-producing pancreatic β-cells fail to compensate for this peripheral insulin resistance, as a result of β-cell dysfunction and death. Long-acting analogs of the incretin hormone GLP-1, such as exendin-4, have been developed for the treatment of type 2 diabetes. We have previously demonstrated that incretin analogs protect frataxin-deficient β-cells from apoptosis in vitro. Our aim was to evaluate the metabolic impact of exendin-4 treatment of regular chow and high fat-fed frataxin-deficient knockin-knockout (KIKO) mice.

**Materials and methods**: Male KIKO and wild type (WT) mice (7-10 animals/group) were placed on regular chow (10% fat) or high fat diet (60% fat) at the age of 12 weeks. 15 weeks later, mini-osmotic pumps were implanted subcutaneously to continuously administer exendin-4 (10 mg/kg/day) or vehicle for 6 weeks, while maintaining the dietary intervention. Food intake and body weight were recorded weekly. Glucose metabolism was examined before and after the 6-week treatment using intraperitoneal glucose tolerance tests (IPGTT) and insulin tolerance tests (ITT). In the IPGTT, blood was collected at 0, 15, 30, 60, 90 and 120 minutes. Glucose tolerance was calculated as the area under the curve of glucose excursions from 0 to 120 minutes. Acute insulin secretion was calculated in the first 15 minutes of the IPGTT. β-cell function was calculated as insulin secretion corrected for insulin sensitivity.

**Results**: After 21 weeks on diet, chow-fed KIKO mice were leaner than WT mice but insulin resistant ($p<0.01$). KIKO mice tended to have reduced β-cell function, but glucose tolerance was not different between genotypes. High fat diet increased fasting glycemia by 30-50 % and impaired glucose tolerance in KIKO and WT mice increasing the glucose excursions by 55% and 95% at the first (11 weeks), and second assessment (21 weeks) respectively, ($p<0.001$). High fat feeding induced insulin resistance in WT mice ($p<0.001$) such that insulin sensitivity was similarly reduced in KIKO mice in either diet, and high fat-fed WT mice. Exendin-4 improved the glucose tolerance of both genotypes on high fat diet by 29% ($p<0.01$), and tended to increase the β-cell function of KIKO but not WT mice. Interestingly, exendin-4 improved glucose tolerance of chow-fed KIKO mice by 22% ($p<0.05$), and improved their insulin secretion and β-cell function by 3- and 2-fold, respectively ($p<0.05$).

**Conclusions**: The KIKO mouse reproduces the insulin resistance and β-cell dysfunction of FRDA patients. Exendin-4 improves glucose tolerance of high fat-fed KIKO and WT mice. Interestingly, exendin-4 also improves glucose tolerance, insulin secretion and β-cell function in chow-fed KIKO mice. Based on these animal data, incretin analogs should be considered as a novel strategy to prevent diabetes in FRDA patients.

**P071**

## Targeting GAA-repeat region with oligonucleotides for the treatment of Friedreich's ataxia

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Friedreich's ataxia (FRDA) is a recessively inherited neuromuscular disorder that arises due to cellular depletion of frataxin (FXN) protein and resulting defects in mitochondrial functions. The protein coding sequence of FXN is normal in the majority of FRDA patients, suggesting that upregulation of endogenous FXN expression could be an effective therapy. The most common molecular cause of this disease is the expansion of GAA/TTC triplet repeats in the first intron of FXN gene. Repeat expansion beyond a certain threshold causes transcriptional defects which reduce FXN mRNA and protein levels. Despite long-standing research in the pathogenesis of FRDA, the means by which GAA-repeat number elevation leads to transcriptional silencing is not clear. DNA-DNA and DNA-RNA interactions formed in the long triplet repeat stretches, defects and alterations in splicing patterns and the formation of a heterochromatin-like structure are among the hypotheses being considered. In order to gain clues into the mechanisms responsible for the FXN deficit in FRDA, we undertook genome-wide analyses to examine the global and local RNA species and chromatin structure and composition changes in FRDA patient cells. Epigenetic screens identified two chromatin modifying complexes as being important in establishing and/or maintaining repeat expansion-induced transcriptional repression at the FXN locus. We identified a novel putative non-coding RNA (ncRNA) potentially responsible for directing the localized epigenetic silencing of the FXN gene. To target this novel ncRNA, we have used locked nucleic acid "gapmer" oligonucleotides consisting of LNA ends and a central DNA stretch to degrade the putative ncRNA by an RNase H-mediated process. Targeting this ncRNA led to FXN mRNA and protein upregulation at therapeutically significant levels in FRDA patient cells in vitro and a FRDA mouse model. The oligonucleotide-based therapeutic approaches developed here pave the way towards the design of multiple strategies for the treatment of FRDA and may have applications for the treatment of other human diseases.

**P072**

## Delivery of the 135 Kb human frataxin genomic DNA locus gives rise to different frataxin isoforms

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Friedreich´s ataxia (FRDA) is the most common form of hereditary ataxia among the Caucasian population and is caused by recessive mutations in the FRDA (FXN) gene which codes for the protein frataxin.

Frataxin is mainly localised in the mitochondria. However, recent results have indicated the presence of different isoforms due to alternative gene expression mechanisms. Thus, in addition to the canonical FXN I isoform (which is localised in the mitochondria), two novel isoforms have been characterised: FXN II, which is mainly found in the cytoplasm, and FXN III which is localised in the cell nucleus. Interestingly, these novel isoforms appear to be expressed in tissues such as the cerebellum and heart, which are severely affected in FRDA.

Our previous studies demonstrated the advantages of using high-capacity herpes simplex virus type 1 (HSV-1) amplicon vectors containing the entire FXN genomic locus (iBAC-FXN) as a gene-delivery vehicle capable of ensuring physiologically-regulated and long-term persistence of FXN gene expression.

Here we describe how expression from the 135 Kb human FXN genomic locus produces the three frataxin isoforms both in cultured neuronal cells and also in vivo after the intracranial injection of an iBAC-FXN into the adult mouse cerebellum. Moreover, we also observed the correct expression of these frataxin isoforms in patient-derived cells after delivery of the iBAC-FXN.

Overall, these results lend further support to the potential use of high-capacity vectors containing entire genomic loci whose expression is mediated by complex transcriptional and posttranscriptional mechanisms for gene therapy applications.

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**P073**

## Frataxin gene delivery using a modular system for installing large transgenes into human-compatible HSV-1 vectors

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Recently, in cancer pain therapy, recombinant non-replicative HSV-1 vectors have successfully reached phase 2 clinical trials for gene delivery into the dorsal root ganglia of the spinal cord via non-invasive intradermal injections (David Fink, University of Michigan). The vector (called NP2) used demonstrates an excellent safety profile even at high doses, and offers an excellent platform for frataxin gene delivery to dorsal root ganglia, which are among the first sites of neurodegeneration in FRDA. However, the NP2 vector cannot accommodate the full *FXN* genomic locus, which we have previously used in preclinical experiments to achieve persistent physiological expression in the adult mouse nervous system.

We have now generated new recombinant non-replicative HSV-1 vectors similar to NP2 with substantially increased transgene capacities while retaining similar safety and large scale production properties. Our current vector version permits the easy installation of large transgenes up to 23 kb: as proof of concept we present our results on a 23 kb reduced *FXN* transgene containing all the genomic elements predicted necessary for longterm physiologically regulated expression.

## P074

### *In vivo* testing of riluzole as a therapeutic for spinocerebellar ataxia type 3

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Spinocerebellar ataxia type 3 (SCA3), also known as Machado-Joseph disease (MJD), belongs to the group of polyglutamine diseases as it is caused by the expansion of a CAG repeat within the *MJD1* gene encoding a polyglutamine repeat in the protein ataxin-3. Up to now, no treatment is available for this disease.

Riluzole (benzothiazol, Rilutek) has been approved for treatment of patients with amyotrophic lateral sclerosis (ALS). In a recent study, a positive effect of riluzole, was observed in a heterogeneous group of patients suffering from different types of cerebellar ataxias after just eight weeks of treatment with riluzole. However, in the mentioned study, only short-term effects were analyzed which may be just symptomatic and no SCA3 patient was included.
In order to analyze whether riluzole may also be beneficial for SCA3 and whether also positive long-term effects of riluzole treatment can be observed, we treated our recently generated mouse model of SCA3 with riluzole. This mouse model allows us to measure a possible effect of riluzole on disease progression. We started the treatment once significant deficits in the rotarod performance were obvious in the generated mice (at 3 months of age) and extensively followed the outcome of the treatment on the behavioural phenotype using e.g. rotarod and measurement of home cage activity for 10 months. Mice were sacrificed at different time points and brain tissue was analyzed for neuropathological alterations, inclusion bodies and the expression level of ataxin-3.

As we could not prove a beneficial effect of riluzole in our SCA3 mouse model, we cannot suggest the use of riluzole for the treatment of SCA3 patients. We, however, successfully established an infrastructure and provided valuable insight into future in vivo testing of promising compounds in SCA3 mouse models.

**P075**

## Mechanism of action of 2-aminobenzamide HDAC inhibitors in reversing frataxin gene silencing

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The genetic defect in Friedreich's ataxia (FRDA) is the hyperexpansion of a GAA·TCC triplet in the first intron of the FXN gene, encoding the essential mitochondrial protein frataxin. Histone posttranslational modifications near the expanded repeats are consistent with heterochromatin formation and consequent FXN gene silencing. Using a newly developed human neuronal cell model, based on patient-derived induced pluripotent stem cells, we find that 2-aminobenzamide histone deacetylase (HDAC) inhibitors increase FXN mRNA levels and frataxin protein in FRDA neuronal cells. However, only compounds targeting the class I HDACs 1, 2 and 3 are active in increasing FXN mRNA in these cells. Structural analogs of the active HDAC inhibitors that selectively target either HDACs 1 and 2 or HDAC3 do not show similar increases in FXN mRNA levels. Combinations of HDAC inhibitors are also ineffective, suggesting some property that is unique to the class I HDAC inhibitors. To understand the mechanism of action of these compounds, we probed the kinetic properties of the active and inactive inhibitors, and found that only compounds that target HDACs 1 – 3 exhibited a slow-on/slow-off mechanism of action for the HDAC enzymes. HDAC1/2 and HDAC3 selective compounds did not show this activity for each member of the class I HDACs. We also used shRNA silencing of HDACs 1, 2 and 3 in the FRDA neuronal cells, with the finding that silencing of each HDAC enzyme leads to increases in FXN mRNA. Based on the known interactions between HDACs in neuronal cells, it is likely that inhibition of each of the class I HDACs is necessary for activation of FXN mRNA synthesis, as there appears to be redundancy in the silencing mechanism caused by the GAA·TCC repeat sequence. Moreover, inhibitors must have a long residence time on their target enzymes for this activity. Our results shed light on the mechanism whereby HDAC inhibitors increase FXN mRNA levels, and point to class I HDAC inhibitors as therapeutics for FRDA.

## P076

### Elucidating the reversibility of ataxia as a basis for development of therapies

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Dominant heterozygous mutations as well as a recently identified homozygous nonsense mutation in the SPTBN2 gene, encoding β-III spectrin, are implicated in human cerebellar ataxia. We have found that a mouse model in which β-III spectrin is knocked-out (KO) mirrors the progressive human phenotype of motor incoordination and cerebellar degeneration, indicating loss of β-III spectrin function in disease pathogenesis. We have therefore used a recombinant adeno-associated viral (rAAV) approach to elucidate whether reintroduction of β-III spectrin can halt, alleviate or reverse the disease phenotype. Given the large size of β-III spectrin we first identified whether a fragment of β-III spectrin could mimic full-length protein function. Human Embryonic Kidney 293 (HEK293) cells were transfected with various fragments of β-III spectrin cloned into Purkinje cell specific pL7-mcherry vectors and analysed by immunofluorescence. We found that the C-terminus fragment of β-III spectrin was localised at the membrane and moreover did not exhibit a dominant negative effect on localization of full-length β-III spectrin. The C-terminal fragment of β-III spectrin was also found to have a similar physiological effect as full-length β-III spectrin with both proteins enhancing sodium currents in primary hippocampal cultures. AAV1/2 particles expressing C-terminus of β-III spectrin under the control of the enhanced synapsin promoter and GFP from an internal ribosome entry site were introduced via stereotaxic injection into mouse cerebellum (lobules IX and X). This region was targeted as Purkinje cell degeneration and loss is first observed in the posterior cerebellum of β-III spectrin KO mice. We found that injection of viral particles expressing GFP alone did not impair animals in rotarod or elevated beam tests, however the motor phenotype of β-III spectrin KO mice where C-terminus of β-III was re-introduced was not alleviated. The lack of a behavioural effect is likely due to the low viral titre and small number of Purkinje cells transduced in this study. Currently steps are being taken to increase the titre of viral particles and hence efficiency of Purkinje cell transduction in subsequent trials. Results from studies investigating whether modulation of calcium signalling affects the morphological and/or physiological defects of Purkinje cells lacking β-III spectrin will also be presented.

## P077

### Coordinative training with exergames can improve advanced multisystemic ataxia

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**Background**: Treatment options are rare in degenerative ataxias, especially if presenting in advanced disease stages and with multisystemic disease load. Moreover, wheelchair-bound subjects with ataxia are commonly excluded from current drug treatment trials, thus leaving them without prospects of access to novel treatments. We recently delivered first evidence that whole-body controlled video games ("exergames") can be used as a training tool in degenerative cerebellar ataxia in a mild to moderate disease stage (Ilg et al 2012, Neurology). Here we aimed to investigate whether also subjects with advanced degenerative ataxia and multisystemic disease load can still benefit from this novel type of training.

**Methods**: We examined the effectiveness of a 12-week exergame-based coordinative training in 7 children (age: 16.9±8.6 years) suffering from advanced progressive cerebellar ataxia who were not or barely able to stand without aid (SARA score: 21.5±5.2). Exercises were based on Wii® video-games, played on the Wii®-Balance board, and specifically selected to train upper- and whole-body balance. Training was divided into two consecutive phases, each combining a supervised introductory and a home-based training period. In phase 1, all patients were trained with the same four games which focused on improving upper-body balance. Phase 2 allowed us to adapt the focus and level of training according to each individual's improvements achieved during phase 1. Training effects were assessed by the following outcome measures: ataxia rating scale (SARA); quantitative movement analysis of upper-body balance; individual goal attainment (GAS). For all measures we used an intra-individual control design, that is, taking subjects before training as their own controls. Assessments were performed two weeks before the training phase1 (E1), immediately prior to phase 1 (E2), after phase 1 (E3) and after phase 2 (E4).

**Results**: Significant reduction of ataxia symptoms (SARA, $p=0.04$) as well as improvements in individually selected goals in everyday living (GAS, E2/E4: 1.125 points) were observed after intervention (E4). Movement analysis revealed a significantly reduced lateral sway in sitting with eyes closed (E1/E4, $p<0.02$), indicating an improvement in body perception and postural control.

**Conclusion**: Our results show that children with ataxia might benefit from exegame-based coordinative training with effects that translate into daily living—even if in advanced disease stages and if suffering from multisystemic neurodegenerative disease (including oculomotor and cognitive deficits). This is important, as most treatment trials currently focus mainly on early disease stages and even deny access for ataxia subjects who are already wheelchair based. Our novel training method might hold many advantages for subjects with advanced ataxias, as it is inexpensive, can be easily performed at home and is highly enjoyable.

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**P078**

## Bioconjugation of BBB-shuttles to viral vectors for the gene therapy of Friedreich's ataxia

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The blood-brain barrier (BBB) is the principal pathway of molecular exchange between the central nervous system (CNS) and the rest of the body. As the brain could be considered the most important organ and also fragile, there is an exhaustive control in the crossing of the surrounding compounds. This task is carried out by the BBB.

However the BBB is also a problem for the therapy of diseases affecting the CNS. One common trouble for all of them is that once an effective drug for its treatment is achieved, it cannot cross through the BBB.

In order to solve the BBB challenge, we are working in a general approach using BBB-shuttles to cross this barrier. These molecules are peptides able to cross the BBB through different mechanisms and carry different cargos that cannot cross unaided. The BBB-shuttles designed to cross through receptor-mediated transcytosis allow the transport of huge cargos, like proteins, antibodies or gold nanoparticles[1-3].

Friedreich's Ataxia (FA) is caused by a diminished expression of Frataxin (FXN) in all the cells, but affecting mainly CNS and heart. Gene therapy based on the delivery of a correct copy of the *FXN* gene is envisaged as a promising approach to treat this disease. In this respect the use of vectors able to effectively cross the BBB is crucial for the treatment of the neurological aspects of FA.

We are working in the development of a gene therapy treatment for FA by using modified viral vectors that allow a long and stable expression of the whole gene of FXN[4]. Furthermore the use of these vectors allows a physiologically regulated expression of FXN protein in the infected cells. Despite its advantages, they are not able to cross the BBB and therefore they cannot access the CNS, the principal affected organ by FA, together with the heart. Thus, BBB-shuttles are coupled to these particles, through bioconjugation methodologies, to achieve their access to the CNS.

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*References*

(1) Guixer, B.; Malakoutikhah, M.; Arranz-Gibert, P.; Teixido, M.; Giralt, E. 'A la carte' peptide shuttles: Tools to increase their passage across the blood-brain barrier. *ChemMedChem* 2014, 9, 1594-1601.

(2) Malakoutikhah, M.; Teixidó, M.; Giralt, E. Shuttle-mediated drug delivery to the brain. *Angew. Chem. Int. Ed.* 2011, 50, 7998-8014.

(3) Prades, R.; Guerrero, S.; Araya, E.; Molina, C.; Salas, E.; Zurita, E.; Selva, J.; Egea, G.; López-Iglesias, C.; Teixidó, M.; Kogan, M. J.; Giralt, E. *Biomaterials* 2012, 33, 7194-7205.

(4) Gimenez-Cassina, A.; Wade-Martins, R.; Gomez-Sebastian, S.; Corona, J. C.; Lim, F.; Diaz-Nido, J. *Gene Ther.* 2011, 18, 1015-1019.

**P079**

## Removal of the polyglutamine stretch from the ataxin-3 protein as a treatment strategy for spinocerebellar ataxia type 3

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Spinocerebellar ataxia type 3 (SCA3) or Machado-Joseph disease (MJD) is one of the nine known neurodegenerative polyglutamine disorders caused by a CAG triplet expansion in the coding region of a gene. In SCA3, the CAG repeat expansion is located in the penultimate exon of the ATXN3 gene. The expanded polyglutamine stretch in the mutant ataxin-3 protein causes a gain of toxic function, which over time leads to neurodegeneration in several brain regions. Currently several genetic therapies are under investigation to downregulate mutant ataxin-3 to treat SCA3. However, it has not yet been established whether ataxin-3 has an essential function in the human brain, and strong downregulation could lead to adverse effects. In our research we make use of antisense oligonucleotides (AONs) to modify proteins. Here, AONs can be used to mask exons from the splicing machinery, resulting in exclusion of targeted exons from the transcript and subsequent translation of a modified protein. The major advantage of this exon skipping approach is that normal levels of protein expression are maintained.

In our study we make use of 2'O-methyl modified AONs with a phosphorothioate backbone to induce in frame exon 9 and 10 skipping from ATXN3 pre-mRNA. AON transfections in patient-derived SCA3 fibroblasts resulted in efficient skipping of both exons and formation of a novel shorter ataxin-3 protein lacking the toxic polyglutamine repeat. Repeated in vivo injections of the AONs were performed in the brain ventricle of transgenic MJD84.2 SCA3 mice. Ataxin-3 exon skipping was found throughout the brain after 5 weekly bolus infusions of 100 µg AONs. However, no modified ataxin-3 protein was detected at this time point. Furthermore, some side effects were observed at these dosages for both the functional and control AONs, disallowing additional AON infusions. Nonetheless, preliminary results indicate a beneficial effect on rotarod performance 10 weeks after treatment when compared to control AONs.

Above results suggest a promising AON based therapy for reducing mutant ataxin-3 toxicity in SCA3, although the toxicity profile of the current AONs requires further optimization for application in brain tissue.

**P080**

## Increased expression of frataxin with TALE-VP64 targeting the frataxin promoter

Jaxques Tremblay, Pierre Chapdelaine, Joël Rousseau

*Université Laval, Canada*

Friedreich's ataxia (FRDA) is due to a reduced frataxin expression due to a trinucleotide repeat. It is 30% in patients with 200 GAA repeats and only 5% in patients with 900 GAA repeats 33. However, carriers of this disease produce about only 50% of the normal level of frataxin but do not develop symptoms. We have engineered 12 genes coding for TALE proteins targeting different nucleotide sequences present in the frataxin promoter. Each expression plasmid contains a TALEFrat gene fused with a transcription activator, VP64 under the EF1a promoter, a 2A peptide and an EGFP. When one of these plasmids was transfected alone in human cells, only green fluorescence was detected by FACS indirectly confirming the expression of the TALEFrat/VP64 protein. To identify which of our 12 different TALEFrat/VP64 proteins were able to better induce the expression of the frataxin gene, we have constructed a reporter plasmid containing the proximal region of the frataxin promoter, followed by a minimal CMV promoter and a mCherry reporter gene (pCR3.1 proximal-promoter-frataxin-miniCMV-mCherry). This reporter plasmid was initially transfected in human cells alone. Very few cells expressed the red fluorescence because the promoter was not effective without transactivation by binding factors. When the reporter plasmid was co-transfected in human cells with one of the pCR3.1-TALEFrat/VP64-2A-EGFP plasmids, a much higher number of cells expressed the red fluorescence because the TALEFrat/VP64 attached to the proximal frataxin promoter and induced the transcription of mCherry. The 3 TALEFrat/VP64, which induced the strongest expression of mCherry, were targeting promoter sequences close to each other.

A plasmid coding for TALEFrat#8/VP64 was nucleofected in normal fibroblasts. Using quantitative RT-PCR, we have confirmed in 3 independent experiments that the expression of the frataxin mRNA (relative to GAPDH mRNA) in human cells was doubled or triple by TALEFrat#8/VP64 when results were normalized with cells transfected with EGFP or non-transfected cells. We have also shown that this TALE also increased by 2 folds the frataxin protein in fibroblasts from a FRDA patient. In a recent preliminary result, we have shown that the transfection of TALEFrat#8/VP64 plasmid in YG8R fibroblasts also increases frataxin mRNA (by about 1.4 to 1.9 fold) and protein (by about 1.4 fold). Such increases would be in the therapeutic range (i.e., 50% of normal frataxin level) for many patients. However, for patients that have less than 25% of the normal level of frataxin expression, a further increase of frataxin would be required and this project may permit to obtain higher frataxin increases and thus this would lead to an increased number of FRDA patients who would benefit from our therapy.

**Friday 27th March 18:00-19:30**

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**P081**

## Defining the molecular signature of Friedreich's ataxia in single cells

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Friedreich's ataxia (FRDA) is an autosomal recessive neurological disease caused by large homozygous expansions of GAA repeat sequences in intron 1 of the frataxin (FXN) gene. FRDA patients homozygous for the GAA expansion have low frataxin mRNA and protein levels when compared with heterozygous carriers and healthy controls. Frataxin is a mitochondrial protein involved in iron-sulfur cluster synthesis, and many of the overt FRDA phenotypes result from deficiencies in various aspects of cell metabolism due to lowered expression of FXN. At the present time there is no effective treatment for Friedreich's ataxia, and easily detectable biomarkers have not been identified. Discovery of FRDA biomarkers is critical in order to more easily monitor patients for disease progression, to implement timely enrollment of patients in clinical drug trials, and to evaluate the effects of potential therapeutic approaches. We conducted RNA sequencing experiments to profile the transcriptomes of 18 FRDA fibroblast cell lines and 17 unaffected control fibroblast cell lines. We found approximately 800 differentially expressed genes between the two groups. In the FRDA patient samples, we identified several differentially expressed genes, not previously associated with FRDA, that may be functionally linked with the cellular pathophysiology of the disease, including SPON1 (reelin signaling), ITPR3 (mitochondrial:ER Ca2+ signaling), and AGRN (neuromuscular junction formation). Furthermore, the expression of these genes correlates well with FXN expression in both controls and patients ($R > |0.5|$). We performed gene expression analysis at the single cell level to determine if the observed changes in gene expression of these candidate genes within the population of cells correlates with changes in FXN expression in individual cells. Single cell analyses of gene expression will help define new mechanisms of disease pathogenesis that might otherwise be masked by similar types of analyses conducted on entire cell populations. Moreover, these analyses are critical for the discovery and validation of disease biomarkers as well as evaluation of the efficacy of therapeutic approaches aimed to treat FRDA patients.

**P082**

## Hypertrophic cardiomyopathy in pediatric Friedreich's ataxia: early left ventricular dysfunction in echocardiographic assessment

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**Introduction**: Cardiac associations with Friedreich's Ataxia (FRDA) are hypertrophic and dilated cardiomyopathies, ventricular dysfunction and arrhythmias. Advanced echocardiopraphic assessment with strain could be a potential marker for early left ventricular dysfunction despite normal left ventricular ejection fraction even in early stages in children.

**Methods**: Retrospective analysis in 14 patients with FRDA were performed. ECG, 24h-cardiac Holter and echocardiograms of all patients with FA were analyzed.

We registered global LV systolic function using Simpson's method and for regional wall motion abnormalities using advanced CMQ (QLAB Philips®) for the longitudinal and circumferential strain analysis.

**Results** : Epidemiology: 75% were boys (median age 7 years). We found data for assessment of global ventricular function in 14 patients and advanced functional assessment with strain in 10 patients. No sudden death in pediatric age was registered in our center. No syncope or chest pain was registered in the clinical reports.

Echocardiography: hypertrophic cardiomyopathy was detected in 12/14 (85%), one of them hypertrophic obstructive cardiomyopathy with mild-to-moderate mitral regurgitation. All cases of hypertrophic cardiomyopathy had threshold levels or a decreased diastolic dysfunction. All of cases had a normal ejection fraction using Simpson's method (58%-79%). One with mild hypertrophy, a regional dysfunction was detected when the longitudinal strain was performed. In all cases of hypertrophy cardiomyopathy, longitudinal strain analysis showed a reduction with segmental variation that was not consistent to a particular region.

ECG: we detected T wave changes in all cases with hypertrophic cardiomyopathy, deep S wave and high R wave in leads V2-V4, and incomplete right branch block in two cases. PR and QTc intervals were normal.

Arrhythmias: two patients with hypertrophic cardiomyopathy had asymptomatic atrial extrasystole registered in the 24h-cardiac Holter. No other arrhythmias had been detected.

Treatment: all cases were under treatment with idebenone or idebenone plus vitE/CoQ10/beta-blocker.

**Conclusions**: Children with FRDA and hypertrophic cardiomyopathy had an asymptomatic diastolic disfunction and segmental disfunction detected with longitudinal strain with a normal ejection fraction.

Seems to be important an advanced assessment of children with FRDA in order to detect this dysfunction progression early.

Whether there is clinical or prognosis value of any of these Strain measurements in FRDA is unknown at this time.

**P083**

## Cardiac involvement in late onset Friedreich ataxia

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Friedreich's ataxia (FRDA) is commonly associated with cardiac structural and functional abnormalities and cardiac disease is the most common cause of mortality. The most common cardiac finding in FRDA is increased left ventricular (LV) relative wall thickness (RWT) due to a combination of increased wall thickness and reduced cavity size, but LV dilatation and reduced ejection fraction(EF) can also develop in this population. Late onset Friedreich ataxia (LOFA), defined as the onset of neurologic symptoms after the age of 25 years, is a less common variant of FRDA and is associated with shorter GAA1 repeats. While there is some data which suggests that LOFA may not be associated with cardiac abnormalities, reported numbers have been relatively small. To further investigate the question of whether there may be cardiac involvement in LOFA we identified 21 subjects with LOFA who had undergone electrocardiography (ECG) and echocardiography at our institution. One of these subjects was excluded because they had a history of myocardial infarction which preceded their initial echocardiogram. Clinical follow-up following the initial echocardiogram varied from 0 to 13 years. The median age of the 20 subjects was 38 years (range of 28-81 years), 11 were males, the age of symptom onset was 28 years (25-57 years) and the symptom duration was 10 years (0-42 years). Median GAA1 was 363 repeats (56-647) and GAA2 was 942 repeats (131-1088). Common ECG abnormalities were voltage criteria of left ventricular hypertrophy, anterior ST elevation and T wave inversion. Only two subjects had a normal ECG. The LV end-diastolic diameter index (LVEDDI) was 2.61±0.26 cm/m2 and 2/20 had dilated ventricles (>3.1 cm/m2). The RWT was 0.40±0.08 and it was elevated (>0.42) in 8/20 subjects. LV mass index was 92±20 g/m2 and it was elevated above the sex-adjusted normal range in three subjects. The EF was 73±9% and 2/20 had a reduced EF (<55%). Nine subjects did not have any abnormal echocardiographic findings including the two subjects with normal ECGs. There were no differences in GAA1, GAA2, age of onset or symptom duration between the groups with normal and abnormal echocardiographic findings ($p>0.05$ for all). One of the subjects with a reduced EF died due to progressive cardiac disease, having also had a cardioembolic stroke. The other subject with a reduced EF has not had either progression or resolution of the LV dysfunction during serial follow-up over 13 years. One subject with a normal EF had an episode of atrial fibrillation which was complicated by systemic thromboembolism. In conclusion, abnormalities of cardiac structure and function and cardiac complications occur in LOFA and while these appear to be less frequent than in subjects with earlier onset disease, cardiac screening is justified in this group.

## P084

### Examination of spasticity and weakness in individuals with Friedreich ataxia

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**Background:** Despite the potential negative impact on mobility and upper limb function there have been few studies examining the prevalence of spasticity in the upper and lower limbs of people with Friedreich ataxia (FRDA). These two cross-sectional studies sought to examine spasticity in the ankle and hands in individuals with FRDA. In addition, the first study examined muscle length changes around the ankle. The latter study also examined the presence of structural changes potentially related to muscle weakness and spasticity in the hands of individuals with FRDA.

**Method:** The Modified Tardieu Scale (MTS) was used to examine the presence of spasticity in the gastrocnemius and soleus muscles in 31 individuals with FRDA. Differences between ambulant (n=18) and non-ambulant (n=13) participants were examined. Relationships between spasticity variables and clinical markers of disease and functional status were also analysed. The MTS, the Modified Ashworth Scale (MAS), testing of muscle strength and documentation of joint range via goniometry was used to examine upper limb function in 15 individuals with FRDA. Relationships between clinical measures of disease severity, and upper limb outcome measures were explored.

**Results**: In the lower limbs all participants had spasticity in at least one muscle examined, and 39% of ambulant and 69% of non-ambulant participants had contracture in one or both of their gastrocnemius muscles. Moreover, significant negative correlations were found between gastrocnemius and soleus angle of catch and the Friedreich Ataxia Rating Scale (FARS) score. The Functional Independence Measure score also demonstrated significant correlations with muscle length, angle of catch and spasticity angle. In the upper limbs 73% of participants demonstrated spasticity in wrist and finger flexors, 46% of participants had contracture in at least one joint in their hands, 86% of participants demonstrated weakness in the intrinsic musculature of the hand and all participants demonstrated some degree of hyperextension and instability at the metacarpopahlangeal joints. The angle of catch ($r=-0.57$, $p<0.05$), spasticity angle ($r=-0.62$, $p<0.05$), MTS spasticity rating ($r=-0.80$, $p<0.01$) and the MAS score ($r=-0.56$, $p<0.05$) at the wrist were all significantly correlated with GAA1. The MTS spasticity rating scale at the wrist also correlated with age at disease onset ($r=-0.61$, $p<0.05$).

**Conclusion**: These studies highlight for the first time the significant incidence of lower limb and upper limb spasticity in people with FRDA. Importantly, spasticity in the gastrocnemius and soleus muscles was evident early in the disease, as well as in ambulant participants. Evidence of both spasticity and weakness in the upper limbs suggests a multifactorial source of functional decline. Upper and lower limb management in people with FRDA should be considered at disease onset to reduce the negative impact of spasticity and weakness and thus optimise function.

**P085**

## Performance on an antisaccade task indicates deficits in task switching capacity in individuals with Friedreich ataxia

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**Background**: The cerebellar dysfunction in Friedreich ataxia (FRDA) has known effects on motor function. It has been suggested that people with FRDA may also have impairment in cognitive capacity either because of direct pathology and/or due to disruption in neural connection between the cerebellum and cerebrum via the thalamus. The ocular motor system is increasingly viewed as a model motor system, with a growing number of studies applying saccadic eye movements as an experimental tool to gain insights into the processes that govern motor control and the cognitive processes underlying goal directed behavior. Examination of the cognitive control of eye movements provides an ideal method to further understand the cognitive impairment associated with FRDA. Task switching is the ability to dynamically adapt behavior to a changing behavioural context and is supported by the frontoparietal networks. We used an antisaccade eye movement task requiring suppression of a reflexive saccade response to examine task switching as a measure of cognitive control in individuals with FRDA. We hypothesised that individuals with FRDA would demonstrate impairment in task switching reflecting a failure to access prefrontal, frontal and parietal regions necessary for effective cognitive control.

**Method**: Twelve individuals with FRDA and eight matched control participants were required to either look toward a peripheral target (prosaccade trial) or away from the target to its mirror location in the opposite visual hemifield (antisaccade trial). Eye movements were recorded with infra-red video oculography using an SR Research Eyelink 1000. Errors and switch cost (increase in saccade latency for switch relative to repeat trials) was calculated.

**Results:** There was a significant difference ($F(1,19) = 5.21$, $p<0.005$) between individuals with FRDA ($M= 17$, $SD = 12$) and controls ($M=7$, $SD = 5.4$) in the number of directional errors. There was also a significant difference ($F(1,18) = 5.92$, $p < 0.05$) between control participants and individuals with FRDA in saccade latency switch cost. Surprisingly, the change in saccade latency to switch trials was less in individuals with FRDA ($M=1.5$, $SD=6.8$) than control participants ($M=14.3$, $SD=16.3$) perhaps indicating impairment in adaptive slowing to allow for cognitive control processes that facilitate effective and accurate task switching.

**Conclusion**: Compared with control participants, people with FRDA demonstrated significant disruption to task switching capacity consistent with reduced inhibitory control and retention of stimulus-response relationships in an antisaccade task. This task places demand on cerebro-ponto-cerebello-thalamo-cerebral loops accessing frontal cortex regions. We propose that in FRDA the function of these loops is impaired, reflecting a failure to access prefrontal/anterior cingulate regions necessary for effective execution of this task.

## P086

### Audio-feedback reduces postural sway in patients with degenerative ataxia

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**Background:** Balance control depends on multi-sensor information and its integration in the central nervous system (CNS). Previous studies have shown that healthy subjects as well as patients suffering from vestibular loss or from Parkinson's Disease can use augmented sensory information like audio-feedback of trunk acceleration to reduce postural sway in standing and walking. However, these findings have never been transferred to patients with cerebellar dysfunctions, e.g. degenerative ataxia. This condition is intrinsically characterized by balance deficits and increased postural sway. However, as the cerebellum is known to be involved in sensor integration, it remains particularly uncertain whether subjects with cerebellar damage can still exploit these additional feedback signals to improve postural control and reduce truncal sway.

**Methods**: We designed a learning paradigm testing for short-term improvements of standing. Subjects had to exploit acoustic feedback signals delivering acoustic information via headphones about the trunk sway of the subject while standing. Successive improvements while standing were assessed in four consecutive phases.1. *Baseline*: standing without audio-feedback; 2. *Audio-feedback*: standing with audio-feedback. 3. *Audio-feedback training while exergaming*: getting more familiarized with the audio-feedback delivered while playing a balance game with Xbox Kinect. 4. *Post-training*: standing with and without feedback to evaluate the post-training benefit of the audio-feedback. Truncal sway at baseline and post-training were assessed with eyes open and eyes closed. This training paradigm was performed in a group of N=18 patients with degenerative cerebellar ataxia. We captured postural sway as a displacement of the centre of gravity using a movement analysis system.

**Results**: After training, patients could significantly reduce the displacement of the centre of gravity in trials using audio-feedback compared to baseline without audio-feedback when having to critically rely on non-visual information (eyes closed) ($p=0.03$). There was no significant difference ($p=0.36$) comparing non-feedback trials before and after the training phase, indicating that the observed improvements were not simply due to prolonged standing time per se, but indeed due to exploiting the acoustic feedback.

**Discussion**: Patients with degenerative ataxia show a significant reduction of postural sway using audio-feedback when having to critically rely on non-visual information, i.e. when having their eyes closed. These findings implicate that, despite progressive degeneration of the cerebellum as a multi-sensor integrator and motor learning machinery, patients with cerebellar dysfunctions still have the ability to exploit augmented sensory information. Thus, audio-feedback might represent a useful tool for future rehabilitation strategies and supporting balance control in cerebellar disease.

1. Dozza M, Horak FB, Chiari L. Auditory biofeedback substitutes for loss of sensory information in maintaining stance. Exp Brain Res 2007;178(1):37-48.

**P087**

## A preliminary longitudinal investigation into cognition and disease progression in spinocerebellar ataxia types 1,2,3,6 and 7

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**Background**: The natural history of clinical symptoms in the spinocerebellar ataxias (SCA)s has been well characterised. However so far there have been no longitudinal studies describing cognitive changes in the SCAs over time. The present study provides a preliminary longitudinal characterisation of the clinical and cognitive profiles in patients with SCA1, SCA2, SCA3, SCA6 and SCA7, with the aim of elucidating the role of the cerebellum in cognition.

**Methods**: 14 patients with different SCAs all caused by CAG repeat expansion (SCA1: n=2; SCA 2: n=2; SCA3 n=3; SCA6 n=4 and SCA7 n=3) completed a comprehensive battery of cognitive and mood assessments at two time points, a mean of 7.35 years apart. All patients were evaluated clinically using the Scale for the Rating and Assessment of Ataxia (SARA) and the Inventory of Non-Ataxia Signs (INAS). Patients underwent structural MRI imaging at time 2.

**Results**: SARA scores at time 2 revealed an increase in ataxia symptoms in all but one SCA6 patient. Changes in non-ataxia signs were more variable, with most increase observed in two SCA3 patients. New impairments on neuropsychological tests were most commonly observed in executive functions, speed and attention and visual memory. Some deterioration was also noted in verbal memory and general intellectual functioning. Results suggest possible differences in SCA subtype progression, with the most rapid cognitive decline apparent in the SCA1 patients and the least in the SCA6 patients. Minimal changes in mood were observed.

**Conclusion**: As well as increasing physical impairment, cognitive decline over time appears to be a distinct aspect of the SCA phenotype. No clear linear relationship between cognitive decline, mood disturbance and disease severity was identifiable, which may suggest a distinct pathology for cognitive and motor performance, unaccounted for by mood.

**P088**

## Alteration of oxidant stress gene expression in Friedreich's ataxia lymphoblast: biomarker potential

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Finding good cellular and molecular biomarkers for Friedreich's ataxia (FA) is important for the study of clinical effectiveness of novel drugs. For example, one's confidence that a drug reactivates silenced frataxin would be increased by a reversal of Friedreich's-specific biomarker expression. Since deficient oxidative stress response has been noted in FA, we decided to investigate the expression of 84 genes involved in oxidative stress, signaling and protection (Oxidative Stress RT2 Profiler PCR Array, Qiagen). We chose to do this in FA lymphoblasts because multiple lines with different GAA expansions are available and they are readily available from patients undergoing clinical testing. Multiple biological replicates were carried out with patient sample of GAA repeat ranging from 460 to 1122. Several antioxidant genes were inhibited in FA lymphoblasts, including mitochondrial superoxide dismutase MnSOD, mitochondrial peroxiredoxin PRDX5, cytosolic PRDX2, and SFTPD. The expression of multiple of these underexpressed genes has been shown to be NRF2-dependent. In terms of induced genes, myoglobin (MB) was strongly and significantly induced, suggesting an active hypoxia response in FA lymphoblasts. The frataxin-dependence of these potential lymphoblastoid biomarkers is being tested experimentally through frataxin gene transfection and frataxin inducing drug treatment, and a comparison of differential expression of these biomarkers in patient fibroblasts and animal models is also planned. If the expressions of these markers are consistent and general across cell types, these could be considered as important biomarkers in support of Friedreich's ataxia therapeutic strategies.

**P089**

## A study of urinary, bowel and sexual function in a large cohort of Friedreich's ataxia patients

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**Background:** Friedreich's Ataxia (FRDA) is an autosomal recessive neurodegenerative disorder which leads to ataxia, weakness, peripheral neuropathy, diabetes and cardiomyopathy. Although patients also report urinary, bowel and sexual symptoms these have not been well described in the literature. We decided to explore this in a large cohort of patients with FRDA.

**Aims:** To evaluate the relationship of urinary, bowel and sexual symptoms with validated disease rating scales in FRDA as well as clinical and genetic features.

**Methods:** 59 Patients seen in a research clinic between April 2013 and February 2014 were included. Questionnaire scores measuring urinary, bowel and sexual symptoms were compared with validated measures of disease severity.

**Results:** Urinary symptom scores correlated significantly (p=0.021) with duration of disease symptoms and spasticity scores (p=0.045). Quality of life measures of patients with urinary symptoms correlated with ataxia rating scales (SARA) (p=0.036) and non-ataxic symptoms (INAS) (p=0.003). Neurogenic Bowel Disease scores correlated significantly with severity of ataxic symptoms (SARA) (p=0.024) and activities of daily living (ADLs) (p=0.001). Sexual symptom scores did not correlate with any established measures of disease severity or disease duration.

**Conclusions:** Urinary symptoms can have a severe impact on patients with FRDA. These correlate with disease duration due to spasticity. These symptoms and their impact on quality of life also correlate with established measures of disease severity. Further studies are necessary to study urinary dysfunction with urodynamic investigations. FRDA patients also need to be monitored for symptoms of bowel dysfunction as these correlate with markers of disease severity. Symptoms of sexual dysfunction did not correlate with any other measured variables perhaps due to the complex multi-factorial physiological and behavioural elements that contribute to them.

**P090**

## Chasing blood-based transcriptional biomarkers for spinocerebellar ataxia type 3 (SCA3): results from a candidate study

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Spinocerebellar ataxia type 3 (SCA3), also known as Machado-Joseph disease (MJD; MIM#109150; ORPHA98757) is a protein misfolding-associated disease, being the worldwide most prevalent spinocerebellar ataxia. Abnormal conformation of mutated ataxin-3 promotes a toxic gain of function, compromising several cellular mechanisms, namely transcription. Whereas SCA3 remains an untreatable disorder, disease-modifying compounds have begun being tested in the context of clinical trials. The success of such trials is largely dependent on the sensitivity of the methods used to measure potential subtle therapeutic benefits, making the search for molecular biomarkers a priority. Alterations in the levels of several proteins have previously been reported in animal and cellular models of SCA3, as well as in patient's samples. Starting from a literature search, 23 genes encoding for such proteins were listed. From these, 7 genes which consistently showed the same pattern of dysregulation in data from a preliminary whole-genome expression microarray performed by our group were selected. Aiming to confirm in a larger set of patients whether these genes would display the same dysregulation pattern and to study their potential as transcriptional biomarkers of SCA3, we used quantitative real-time PCR (qPCR); expression levels of *DNAJB1, HSPB1, DNAJB14, DNAJB12, BAX, BCL2* and *SOD2* were measured in blood of SCA3 patients and controls. *HSPB1, DNAJB12* and *BCL2* were found to be significantly dysregulated ($p<0.05$). *DNAJB14* and *BAX* transcript levels significantly correlated with disease duration, with patients closer to the age at onset presenting lower expression levels ($p=0.001$ and $p=0.014$, respectively). Moreover, *BAX* expression levels were also positively correlated with the age at onset ($p=0.037$). Longitudinal studies, using samples of the same SCA3 subjects collected at distinct moments of disease progression, are warranted to confirm the potential of these genes as transcriptional biomarkers of SCA3 progression.

**P091**

## Using smartphones to measure and quantify key characteristics of Friedreich's ataxia: a pilot study

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**Objective**: To advance the study of functional and home-based measures in Friedreich's Ataxia (FA) through the use of smartphone technology. Specifically, our aim is to test the practicality and effectiveness of cheap, ubiquitous, consumer-grade smartphones to: (1) objectively measure and quantify key characteristics of FA (voice, gait, postural sway, finger tapping and reaction times, and other aspects of daily behaviour), and, (2) discriminate FA participants from healthy controls, using only the smartphone test recordings.

We are conducting an ongoing, controlled study with 30 participants, comprising FA participants and controls. All participants have been provided identical LG Optimus S Android smartphones, which have built-in voice recorders, accelerometers, and touch screens. Using these smartphones, participants are conducting the following self-administered, short tests (less than 5 minute) each day in their home and community settings: (1) (voice test) say the sustained phonation 'aaah' for as long and as steadily as possible; (2) (posture test) stand upright unaided for thirty seconds; (3) (gait test) walk twenty steps forward, turn around, and return back to the starting position; (4) (finger tapping test) tap the screen alternately keeping a regular rhythm; and (5) (reaction time test) press and hold the on-screen button as soon as it appears and release it as soon as it disappears. Furthermore, these smartphones also passively record sensor time traces continuously in the background. From the smartphone data, we intend to extract a wide range of summary measures, which could be used to quantify fine-grained changes in symptoms over time. Using machine learning techniques, we would then identify discriminating patterns in the summary measures that could potentially be used to distinguish FA participants from controls.

**Results**: At the time of submission, 9 participants have enrolled for the study. Participants have each contributed an average of 46 self-administered tests and an estimated 140 hours of passively collected, daily behavioural data.

**Conclusions**: Consumer-grade smartphones seem to be a viable means for collecting high frequency data that can potentially quantify and uncover daily variations in key characteristics of FA. Future studies could test the efficacy of this smartphone application and statistical framework in discriminating FA from other disorders that cause changes in voice, impairment in cognitive skills, or result in tremor/gait deficits. We envisage that measuring and quantifying FA characteristics via smartphones is feasible, and that smartphones have potential value as an objective symptom monitoring.

**P092**

## Body habitus is inversely related to neurological disease severity in children with Friedreich ataxia

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**Background**: Patients with Friedreich Ataxia (FRDA) have been proposed to have impaired metabolism and altered body habitus. Children with FRDA tend to be substantially underweight. As FRDA progresses, ambulatory status declines and adults may become overweight or obese. In other progressive neurologic diseases, nutritional status has been correlated to disease severity. This study aimed to determine whether there is a relationship between body habitus and FRDA disease characteristics.

**Methods**: We reviewed cross-sectional data from the CCRN natural history database of 535 patients with FRDA seen at 12 international medical centers . Data was stratified into two categories: children/adolescents (ages 5 to 20, n=246) and adults (ages 21 to 76, n=289). Multivariate regression analysis was performed comparing body mass index (BMI), BMI percentile, or body surface area (BSA) calculations with neurological disease severity, determined by FARS or performance scores controlling for sex, age, and GAA repeat length.

**Results**: In children/adolescents, the mean age was 14, mean GAA repeat length was 713, and 53% were male. In adults, the mean age was 37, mean GAA repeat length was 546, and 50% were male. BMI percentile varied greatly in both children/adolescents (range = 0-99.1, mean =42.4) and adults (range = 0-99.7, mean = 56.3). Among children/adolescents, 17.5% were underweight, while only 4.5% of adults were underweight. Whereas 20% of children/adolescents were either overweight or obese, 33.5% of adults were overweight or obese. Age predicted BMI and BSA, but not BMI percentile, for all age groups.

In children/adolescents, there was an inverse relationship between BMI ($p=0.035$), BMI percentile ($p=0.003$), and BSA ($p=0.001$) with FARS scores, even when accounting for sex, age, and GAA repeat length. These body weight measures also predicted z-scores for timed 25-foot walk and 9-hole peg test. In all regression models, BMI, BMI percentile, and BSA were related to GAA repeat length (all $p<0.001$).

In adults, the regression showed no relationship between body size and disease severity. Body habitus was related to GAA repeat length compared with FARS scores ($p<0.001$) but not with performance measures.

**Conclusion**: This study of a large cohort of FRDA patients shows that GAA repeat length predicts BMI and BSA, suggesting that these body habitus measures are inherent features of the disease phenotype. Our results also suggest that BMI and BSA are independent factors associated with neurologic severity, especially in younger patients, consistent with metabolic factors contributing to both nutritional and neurological alterations in FRDA. These findings emphasize the need for further studies of metabolism and body composition in FRDA. Future studies may be aimed to determine whether improving nutritional status could modify neurological progression in FRDA.

**P093**

## Frataxin levels as a biomarker of disease features in Friedreich ataxia

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**Background**: Friedreich ataxia (FRDA) is an autosomal recessive ataxia resulting from mutations in the frataxin gene (FXN). Such mutations, usually expanded intronic GAA repeats (98%), give rise to decreased levels of frataxin in affected and unaffected tissues. Most pathogenic point mutations (2% of patients) disrupt RNA splicing, translation initiation or protein folding of frataxin and consequently result in minimal functional protein. A few mutations (R165C, W155R, I154F and G130V) are not within the core of the protein, and could give rise to dysfunctional frataxin. Previously, we examined levels of frataxin in peripheral tissues to ascertain their utility as biomarkers of the disease.

**Methods**: In the present study, we increased the sample size to a total of 525 subjects, used repeated measures of subjects, performed correlations with clinical status in multiple tissues, and looked in detail at results from individuals with point mutations to examine more thoroughly the utility of frataxin measurement in buccal cells and blood cells. All samples were assayed using lateral flow assays for frataxin.

**Results**: In both blood and buccal cells, there was no change in frataxin levels over time with repeated measures analysis, although cross-sectional data analysis predicted a very small increase in frataxin levels over decades ($p<0.001$) . Frataxin levels in both tissues were predicted by GAA repeat length ($p<0.001$) and frataxin levels themselves predicted clinical neurological ratings (accounting for age). The associations were stronger in blood than in buccal cells (R2 values two times higher in blood); however, frataxin levels in FRDA buccal cells were substantially lower than blood when compared to control. This suggests that levels in both tissues may have value as biomarkers. Point mutations in FXN were generally associated with lower levels of frataxin than the presence of two GAA repeat expansions, though levels varied dramatically between tissues in some subjects with point mutations. In addition, selected point mutations (R165C, W155R) were associated with normal levels of frataxin, consistent with the concept that such mutations lead to dysfunctional frataxin rather than absent frataxin. Surprisingly, patients carrying G130V mutations, who have a mild, atypical phenotype, had lower levels frataxin compared with patients carrying two GAA repeats in both blood and in fibroblasts (6% control vs 15% control). Subjects with start codon mutations demonstrated marked differences in frataxin level between tissues, having normal levels in blood but extremely low levels in buccal cells.

**Discussion:** The present data show that peripheral frataxin levels reflect disease features in FRDA, but emphasize the need for further analyses and interpretation of such levels in the context of specific mutations. Future studies likely should be interpreted in the context of results from multiple tissues, and potentially include novel, clinically affected tissue types.

**P094**

## Association between plasma mitochondrial DNA and disease duration in Friedreich's ataxia patients

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**Background**: Friedreich's ataxia is the most common autosomal recessive inherited ataxia affecting multiple systems with heterogeneous clinical symptoms. Identification of biomarker for monitoring or prognostic application remains to be a challenging topic. This study aimed to quantify the levels of plasma cell free nucleic acids and to evaluate with the clinico-pathological features of the patients.

**Methods**: Patient's clinical information was assessed using International cooperative ataxia rating scale and disease was diagnosed using Long Range PCR. Levels of frataxin were quantified using ELISA. Plasma nuclear and mitochondrial DNA levels were estimated by Multiplex real time quantitative polymerase chain reaction assay using GAPDH and ATP8 gene markers

**Results**: Absolute quantification of plasma nuclear and mitochondrial DNA in Friedreich's patients and healthy controls revealed significant differentiation, but no significant association obtained with patient pathological features. However, relative quantity of plasma mitochondrial DNA in patients was found to be decreased and inversely correlated ($p<0.05$) with disease duration.

**Conclusion**: The present study suggests relative levels of plasma mitochondrial DNA as a potential marker for the monitoring/prognosis of Friedreich's ataxia patients.

*Ethical Statement: Present work was carried out with the consent of patients and approved by Institutional Ethical Committee (Sanction No: IEC/NP-311/2012/RP-24/2012).Source of Funding: Indian Council of Medical Research of India (5/4-5/85/Neuro-2012-NCD-I) Competing Interests: None.*

**P095**

## Molecular & biochemical assessment and genetic counseling offered to Friedreich's ataxia patients of Indian origin

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**Background**: In India, prevailing lack of molecular diagnostic procedures and limited knowledge of friedreich's ataxia among primary health care providers led to misdiagnosis of friedreich's ataxia due to clinical overlap, and its low prevalence in India attracts least attention by researchers. In this study, we tried to generate data regarding GAA repeat size, antioxidant levels, epigenetic alterations at the upstream of GAA repeat and frataxin protein level from friedreich's ataxia patient of Indian origin, and offering family screening to the patient relative followed by genetic counseling.

**Method**: From 41 suspected patients and 50 controls, we collected 3 ml of blood in EDTA vial. EDTA blood served as source of DNA as well as lymphocytes. Repeat characterization was done by short-PCR, Triplet repeat primed PCR and long range-PCR. Cases confirmed for friedreich ataxia was further evaluated for Frataxin level, oxidative markers, and epigenetic alternation by bisulfite sequencing. In confirmed cases, family members were screened for carrier status or any prospective cases.

**Results**: Suspected subjects (48) were screened by Short-PCR, TP-PCR and long PCR and found 28 cases to be positive. Out of these 28 cases, we were able to convincingly obtain the samples from family members of 5 cases. On screening we found 9 of them as carriers. Genetic counseling was offered to all the members of family who were found carriers for friedreich ataxia. Frataxin protein levels were significantly reduced in patients (0.065 pg/ug of protein) compared with controls (0.265 pg/ug of protein) and carriers (0.188 pg/ug of protein). The oxidative markers – SOD, GPX and GST – levels were elevated in patients compared with controls and carriers but statistical significance was only observed with SOD. No significant observation was found with CpG island methylation at first intron. Other epigenetic studies are in progress.

**P096**

## Neurotechnology biomarkers in Friedreich's ataxia

Constantinos Gavriel, Anastasia Sylaidi, Stathij Nageshwaran, Pedro Lourenco, Romy Lorenz, Andreas Thomik, Richard Festenstein, Aldo Faisal

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Closer monitoring of FRDA allows more accurate assessment of disease progression, the discovery of FRDA sub-types and may provide pragmatic end-points for clinical trials. To this end we have devised and are validating a number of neurotechnology tools, which may aide in diagnosis and disease monitoring.

A motion capture suit has been customised to record a patient's movement at 52 seperate sensors around all body joints, following which SARA, SCAFI and ADL (breakfast, bedroom, office scenarios) assessments are undertaken. The longitudinal use of this tool in a clinical trial setting may highlight subtle changes in disease state following disease modyfing interventions. Principle component analysis on large kinematic data sets will help to provide a FRDA movement fingerprint that can be used to streamline data acquisition from the most informative suit sensors. Assessments have been undertaken over a 1 year period in 9 FRDA subjects and 5 matched controls. Data analysis is currently underway and will be available for presentation at the meeting.

fMRI provides insight into brain areas recruited during motor tasks. To assess differences in motor learning of FRDA patients we have devised an intra-MRI tool manipulation task. 9 patients over 1 year and 5 controls have been assessed. Preliminary analysis has highlighted a clear difference in motor learning between FRDA subjects and age-sex matched controls. This biomarker will be valuable for use in a clinical trial of FRDA.

We will correlate our findings with frataxin protein expression at each time point.

**P097**

## Computational analysis of clinical patient data from the EFACTS project

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Clinical and demographic data has been collected by the clinical partners of the EFACTS EU consortium from over 600 FRDA patients. The data representing 51 different features was collected and analysed from each patient; this data has been analysed using computational data analysis techniques. The objective was to identify which features are the most informative in classifying the patients into different subgroups, and then identifying key stages in the development of the disease and predict disease progression in these patient subgroups. Hill Climbing followed by the J48 algorithm has been used to identify subgroups of patients which differ in their clinical attributes. The strongest classifier of age of onset disease subgroups is the GAA repeat number since any analysis removing this feature decreased the value of kappa (a measure of the accuracy of classification). An age of onset below 18 can be predicted with high accuracy using GAA repeat alone if the GAA repeat is more than 500bp. This indicates the importance of accurate measurement of the number of repeats as a prognosis tool. When patients were manually subdivided into 4 groups according to age of onset, the features that were important as markers of disease progression were identified by classifying which features were most informative at particular time points after the onset of the disease [duration]. These were elements of the SARA score, cardiac and activities of daily living, placing less important on INAS. The informative features differed according to the age of onset showing that the disease can be divided into sub types according to onset age. Interestingly, cardiac features are not informative as indicators in patients with an age of onset above 26 years indicating that these features do not change significantly with time in these patients. The converse was true for patients with an onset of 5 years or younger. To establish if there is a difference in severity of male and female patients we can show that cardiac features are less affected in females than males with a classification accuracy of 64%, with intra ventricular thickness being by far the best classifier, usually being less than 10.3mm in females. When taking into account all the features males and females can be correctly classified 71% of the time indicating a possible difference in how the disease affects males and females. Using Bayesian techniques we are creating reliable time-series models from the cross-sectional data that has been collected using the temporal bootstrap technique to identify different disease states along disease progression trajectories, as well as the transitions between them. Further analyses are being undertaken to predict changes to the parameters and identify the best features for prognosis tools.

**P098**

## Cerebellar immunohistochemical staining patterns in idiopathic sporadic ataxia: more evidence of an autoimmune aetiology?

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**Introduction**: Cerebellar ataxia is a heterogeneous group of disorders that are inherited or acquired. Acquired forms include immune mediated ataxias, chronic alcohol use, toxic effects from phenytoin and degenerative conditions such as cerebellar variant of multi-system atrophy. Immune mediated ataxias include paraneoplastic cerebellar degeneration, post-infectious cerebellitis, gluten ataxia, ataxia with anti-glutamic acid decarboxylase (GAD) antibodies and Primary Autoimmune Cerebellar Ataxia (PACA). The exact immune mediated pathogenic mechanisms responsible remain unclear. Idiopathic sporadic ataxia (ISA) is defined as a late onset progressive ataxia of undetermined aetiology. A proportion of patients with ISA have an autoimmune tendency (e.g. have other autoimmune diseases), possess the HLA DQ2/DQ8 (associated with autoimmune disorders) and have serological evidence of antibodies against cerebellar cells. This group of patients is likely to represent PACA. We used indirect immunohistochemistry to determine if specific cerebellar staining patterns can be identified and help distinguish PACA from ISA.

**Methods**: Forty-eight patients with ISA were recruited from the Sheffield Ataxia Centre, UK. Reactivity of patient sera on adult Sprague-Dawley rat cerebellar tissue was assessed by indirect immunohistochemistry based on a previous published method (Hadjivassiliou et al; 2002). Mouse anti-Calbindin-D-28K monoclonal antibody was used as a positive control. Negative controls included sections incubated without patient sera. We have also previously studied as a disease control patients with genetic ataxias. Secondary antibodies consisted of a horseradish peroxidase-conjugated IgG antibody. Sections were developed with a 3,3'- diaminobenzidine (DAB) substrate kit. Patient and control samples were run simultaneously. Two blinded observers performed semi-quantitative evaluation of the staining intensity independently. Positive staining was recorded if Purkinje cell staining was above background levels.

**Results**: The 1:600 dilution was identified as the optimum sera dilution in determining the reactivity of patient sera on rat cerebellar tissue. There was 77% concordance between the 2-blinded observers. Positive Purkinje cell staining was demonstrated in 17/48 (35%) patients with ISA. This included 13 with weak staining and 4 with strong staining that were comparable to that seen in other immune mediated ataxias. Ten of the 17 patients did have an underlying autoimmune background and 9/17 had HLA DQ2/DQ8. No Purkinje cell staining was seen in healthy controls. Staining of other neuronal cell populations were noted in particular the Granular layer. Strong Granular layer staining was seen in 31/48 (65%) patients. Both Purkinje cell and strong granular layer staining were seen in 12/48 (25%) patients with ISA.

**Discussion**: Specific immunohistochemical staining patterns of Purkinje cell and Granular layers can be identified from sera of patients with ISA. The presence of such staining strengthens the concept of PACA as a disease entity. More detailed immunohistochemical characterisation of patients with ISA may help identify patients with immune ataxia that may be amenable to treatment with immunomodulation.

**P099**

## Force dysmetria in spinocerebellar ataxia 6 correlates with functional capacity

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Spinocerebellar ataxia type 6 (SCA6) is a genetic disease that causes pure cerebellar degeneration affecting walking, balance, and coordination. One of the main symptoms of SCA6 is dysmetria. However, the magnitude of dysmetria and its relation to functional capacity in SCA6 has not been studied. Our purpose was to quantify dysmetria and determine the relation between dysmetria and functional capacity in SCA6. Ten individuals diagnosed and genetically confirmed with SCA6 (63.7 ± 7.02yrs) and nine age-matched healthy controls (65.9 ± 8.5yrs) performed goal-directed isometric contractions with the ankle joint. Dysmetria was quantified as the force and time error during goal-directed contractions. SCA6 functional capacity was determined by ICARS and SARA clinical assessments. We found that SCA6 participants exhibited greater force dysmetria than healthy controls ($P < 0.05$). In contrast, SCA6 participants exhibited lesser time dysmetria than healthy controls ($P < 0.05$). Only force dysmetria was related to SCA6 functional capacity, as measured with ICARS kinetic score ($R2 = 0.63$), ICARS total score ($R2 = 0.43$), and SARA total score ($R2 = 0.46$). Our findings demonstrate that SCA6 exhibit force dysmetria and that force dysmetria is associated to SCA6 functional capacity. Quantifying dysmetria in individuals with SCA6 could provide a more objective evaluation of the functional capacity and disease progression in SCA6. This novel finding may stimulate rehabilitation protocols to improve force dysmetria in SCA6 and consequently improve their ataxic gait and diminished functional capacity.

**P100**

## Quantitative visual assessment and vision related quality of life measures in spinocerebellar ataxia

Sachin Kedar[1], Deepta Ghate[1], Earnest Murray[2], James Corbett[3], S H Subramony[4]

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Spinocerebellar ataxia is a neurodegenerative condition that produces abnormalities of ocular motility and alignment. In this cross-sectional study of subjects with spinocerebellar ataxia, we quantitatively assessed vision, ocular motility and alignment and vision related quality of life. Nineteen genetically diagnosed spinocerebellar ataxia patients (eleven spinocerebellar ataxia type 3, three spinocerebellar ataxia type 1 and five spinocerebellar ataxia type 6) participated at two university centers. All subjects were administered the National Eye Institute-Visual Function Questionnaire, the 10-Item Neuro-Ophthalmic Supplement, scale for the assessment and rating of ataxia score and complete ophthalmic examination. Quantitative assessment of ocular motility and alignment was completed by twelve subjects. The mean age of the study subjects was 56±11 years; disease duration was 9±2 years; scale for the assessment and rating of ataxia score was 16.71±6.29. Spinocerebellar ataxia patients had normal distance visual acuity, near visual acuity and color vision, but showed decreased low contrast sensitivity (1.25%: 19.84±2.6 letters; 2.5%: 28.37±8.58 letters) and stereoacuity (55±22.79 seconds). All twelve patients who underwent detailed ocular motility examination showed abnormalities which included gaze limitation (9/12); nystagmus (5/12); distance esophoria (11/12); near exophoria (12/12); receded near point of convergence (28.7±12.83 cm) and decreased near convergence amplitude (10.67±6.77 prism diopters) compared to established normal ranges. Composite scores for the National Eye Institute-Visual Function Questionnaire (mean 76.3±13; normal 93±13) and 10-Item Neuro- Ophthalmic Supplement (mean 65.2±16.8; normal 93±7) were significantly decreased. Poor National Eye Institute-Visual Function Questionnaire subscale scores were observed for general vision (mean 70.5±13.9; normal 83±15), near vision (mean 74.6±16.1; normal 92±13), distance vision (mean 71.5±21; normal 93±11), driving (mean 42.9±17.4; normal 87±18) and peripheral vision (mean 59.2±27.9; normal 97±10) while normal scores on the general health subscale (mean 59.2±26.6; normal 69±24). Decreased near fusional convergence amplitude was found to correlate with poor scores on a majority of these subscales. We have demonstrated in this cohort of subjects with spinocerebellar ataxia, a significant impairment in vision related quality of life measures that is independent of the severity of underlying condition (as measured by the general health subscale and ataxia severity score measures). Neurologists caring for patients with neurodegenerative conditions should assess their patients for visual disability. Low scores on National Eye Institute-Visual Function Questionnaire and Neuro- Ophthalmic Supplement should prompt neuro-ophthalmic examination.

**P101**

## Robotic and clinical evaluation of upper limb motor performance in patients with Friedreich's ataxia

Qessica Vasco[1, 5], Marco Germanotta[2], Maurizio Petrarca[1], Stefano Rossi[3], Sacha Carniel[1], Paolo Cappa[4], Enrico Bertini[5], Enrico Castelli[1]

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**Background**: Friedreich's ataxia (FRDA) is the most common form of childhood onset ataxia. In this disease there is early manifestation of gait ataxia, and dysmetria of the arms and legs which causes impairment in daily activities that require fine manual dexterity. To date there is no cure for this disease. Some novel therapeutic approaches are ongoing in different steps of clinical trial. Development of sensitive outcome measures is crucial to prove therapeutic effectiveness. The aim of the study was to assess the reliability and sensitivity of quantitative and objective assessment of upper limb performance computed by means of robotic device and to evaluate the correlation with the Scale for the Assessment and Rating of Ataxia (SARA).

**Methods**: Here we assess upper limb performances by means of InMotion Arm Robot, a robot designed for clinical neurological applications, in a cohort of 14 children and young adults affected by FRDA, matched for age with 18 healthy subjects. We focused on the analysis of kinematics, accuracy, smoothness, and submovements of the upper limb while reaching movements were performed. The robotic evaluation of upper limb performance consisted of planar reaching movements performed with the robotic system. The motors of the robot were turned off, so that the device worked as a measurement tool. The status of the disease was scored using SARA.

**Results**: All our robotic indices were significantly different between the two cohorts except for two, and were highly and reliably discriminative between healthy and subjects with FRDA. In particular, subjects with FRDA exhibited slower movements as well as loss of accuracy and smoothness, which are typical of the disease. Duration of Movement, Mean Velocity, Normalized Jerk, and Number of Submovements were the best discriminative indices, as they were directly and easily measurable and correlated with the status of the disease, as measured by SARA.

**Conclusions**: Our results suggest that outcome measures obtained by means of robotic devices can improve the sensitivity of clinical evaluations of patients' dexterity and can accurately and efficiently quantify changes over time in clinical trials, particularly when functional scales appear to be no longer sensitive.

**P102**

## Longitudinal study of balance dysfunction in Friedreich's ataxia using the biodex balance system SD

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**Objective:** To quantify longitudinal changes in balance in Friedreich's Ataxia (FA) patients compared to controls over a 24-month period using the Biodex Balance System SD.

**Background:** FA is a devastating neurodegenerative disease. Objective measures to quantify small changes in neurological deficits in FA patients are needed to facilitate therapeutic clinical trials.

**Methods**: This was a prospective, longitudinal study that evaluated balance in ambulatory FA patients compared to healthy, matched controls. FA patients were examined at baseline and at 6, 12, and 24 months using the Biodex Balance System SD, a posturography system used to assess balance dysfunction, and the Friedreich's Ataxia Rating Scale (FARS). Controls were evaluated at baseline and 12 months. Changes in balance parameters over time were estimated and compared using multilevel modeling. The results generated by this system are presented with the postural stability indices (Overall Stability Index (OSI), Anterior/Posterior Stability Index (API), and Medial/Lateral Stability Index (MLI)), all with either eyes open (EO) or eyes closed (EC).

**Results**: Eight FA patients (aged 29.4 ± 9.0) and 8 controls (aged 29.6 ± 9.1) were included in this analysis. The percent change (monthly change rate) in postural stability indices, indicating increase in instability, for FA patients after 24 months was: OSI EO 31.2%(0.026); OSI EC 54.2% (0.095); API EO 34.1% (0.022); API EC 67.5% (0.080); MLI EO 28.3%(0.012), and MLI EC 41.3%(0.044), while there was essentially no change in these measures among controls after one year. After 24 months, the mean FARS score in FA patients increased 21%.

**Conclusions**: FA patients demonstrated significant changes in postural stability indices, especially with eyes closed, over two years. This suggests that postural stability measures using a specialized equipment are highly sensitive to change in FA and could be important endpoints in future clinical trials."

## P103

### A comparison of three ataxia rating scales in Friedreich ataxia

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**Background:** Friedreich ataxia (FRDA) is an autosomal recessive neurodegenerative condition characterised by progressive ataxia, dysarthria, scoliosis, hypertrophic cardiomyopathy and diabetes mellitus. Current scales used to measure the progression of FRDA include the Friedreich Ataxia Rating Scale (FARS) and the International Cooperative Ataxia Rating Scale (ICARS). The Scale for the Assessment and Rating of Ataxia (SARA) has also been recently validated as a measure of ataxia and neurologic disability in FRDA. Compared to the FARS and ICARS, the SARA has fewer items, is quicker to administer and has high interrater reliability. This study aimed to compare the responsiveness of the three rating scales over a period of three years.

**Method:** One hundred and one individuals homozygous for a GAA expansion in intron 1 of FXN completed the SARA, FARS and ICARS at baseline. Sixty-eight individuals completed all three assessments at Year 1 and forty-eight at Year 2. Fifty-eight percent of the cohort were male, and the average age of onset at baseline was 15.3 years. Pearson product-moment correlation coefficient was used to determine correlations between the three rating scales and various FRDA clinical characteristics, including GAA1 repeat size, GAA2 repeat size and age of disease onset. Higher scores on all three scales indicate worse disease. Paired t-tests were conducted to examine the change of all three assessments at three different time intervals; from baseline to Year 1, from Year 1 to Year 2, as well as from baseline to Year 2.

**Results:** Consistent with previous studies, the SARA correlated significantly with the FARS ($r=0.96$, $p<0.01$) and the ICARS ($r=0.98$, $p<0.01$) at baseline. Age of disease onset correlated significantly with the SARA ($r=-0.27$, $p<0.01$), FARS ($r=-0.27$, $p<0.01$) and ICARS ($r=-0.26$, $p<0.01$); GAA1 repeat size also showed significant correlations with all three rating scales: SARA ($r=0.35$, $p<0.01$), FARS ($r=0.34$, $p<0.01$), ICARS ($r=0.33$, $p<0.01$), as did GAA2 repeat size: SARA ($r=0.39$, $p<0.01$), FARS ($r=0.39$, $p<0.01$), ICARS ($r=0.42$, $p<0.01$).

There were no significant differences found in all three scales scores from baseline to Year 1. The ICARS showed a significant change in scales score from Years 1 to 2 ($t(-2.38)=2.91$, $p<0.05$) as well as from baseline to Year 2 ($t(-2.22)=1.84$, $p<0.05$). There was no significant change in the SARA or FARS from year 1 to year 2, or baseline to year 2.

**Conclusions:** The ICARS was the only rating scale to show a significant change over two years in comparison to the FARS and the SARA. The lack of change reflected in the scores of the FARS and SARA may well reflect lack of power. This study is ongoing and aims to collect data on a larger number of participants longitudinally. This will allow better assessment of the responsiveness of the rating scales over time.

**P104**

## Eotaxin: a candidate biomarker of the progression of Machado Joseph disease/spinocerebellar ataxia 3

Gerson Carvalho[1], Jonas Saute[1, 2], Clarissa Haas[1], Vitor Torrez[1], Andressa Brochier[1], Gabriele Souza[1], Gabriel Furtado[1], Tailise Gheno[1], Aline Russo[1], Thais Monte[1, 9], Artur Schuh[9], Rui D'Avila[2], Karina Donis[2], Raphael Castilhos[1], Diogo Souza[1,5], Maria-Luiza Saraiva-Pereira[1, 2], Vanessa Torman[1], Suzi Camey[1], Luis Portela[1,5], Laura Jardim[1, 2,4]

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**Aims**: to describe the serum concentrations of a broad spectrum of cytokines in symptomatic and asymptomatic carriers of Machado Joseph disease (SCA3/MJD) CAG expansions.

**Methods:** molecularly confirmed carriers and controls were studied. Age at onset, disease duration, and clinical scales SARA, NESSCA, SCAFI and CCFS were obtained from the symptomatic carriers. Serum was obtained from all individuals and a cytokine panel was performed, including eotaxin, GM-CSF, IFN-a, IFN-γ, lL-1b, IL-1RA , IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, IP-10, MCP-1, MIG, MIP1a, MIP1b, RANTES and TNF-a. In a subgroup of symptomatic carriers, the cytokine panel was repeated after 360 days. Cytokines distribution among groups was studied by discriminant analysis; changes in serum levels after 360 days were studied by generalized estimation equation.

**Results**: 66 symptomatic carriers, 13 asymptomatic carriers, and 43 controls were studied. No differences in cytokine patterns were found between controls and all carriers of the CAG expansions, or between controls and symptomatic carriers only. In contrast, eotaxin concentrations were significantly higher in asymptomatic than in symptomatic carriers, or in controls (p=0.001, ANCOVA). Eotaxin did not correlate with age, disease duration, CAG expansion, NESSCA, and SARA. Among symptomatic carriers, eotaxin concentrations fell after 360 days (p = 0.039, GEE).

**Discussion**: SCA3/MJD presents a benign pattern of serum cytokines. In contrast, levels of eotaxin, a peptide secreted by astrocytes, were elevated in the asymptomatic carriers, suggesting that a specific response of these cells can be related to symptom onset and/or progression, in SCA3/MJD.

**P105**

## Dysphagia in Friedreich ataxia

Megan Keage[1], Martin Delatycki[2], Louise Corben[2], Adam Vogel[1]

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**Background and aims**: Dysphagia is common in patients with Friedreich ataxia (FRDA) yet little is known about the degree and nature of these impairments. To date swallowing difficulties have been described in the context of clinical bedside assessments or neurological reports, yet no studies present quantitative data based on current gold standard assessment protocols.

**Methods**: 50 consecutive patients with a genetically confirmed diagnosis of FRDA were recruited from the Friedreich Ataxia Clinic in Melbourne, Australia. Swallowing and oral motor function were assessed via clinical bedside exam, the Frenchay Dysarthria Assessment 2 (FDA-2) and a Videofluoroscopic Study of Swallowing (VFSS). Swallowing related quality of life was determined using the SWAL-QOL questionnaire.

**Results**: All patients with FRDA presented with oropharyngeal dysphagia. 32 patients underwent VFSS, of which 65.63% (n=21) were observed aspirating/penetrating on solids (puree and biscuit) and unmodified fluids. In line with previous research, patients with FRDA also reported reduced quality of life as a consequence of swallowing impairment.

**Conclusion**: Here we provide data comprehensively documenting the nature and impact of dysphagia on patients with FRDA. Findings assist in informing management and therapeutic intervention strategies and establish a context on which to base future clinical trials.

**Friday 27th March 18:00-19:30**

## P106

### An open label clinical pilot study of resveratrol as a treatment for Friedreich ataxia

Eppie Yiu[1, 2, 3], Geneieve Tai[1], Roger Peverill[4], Katherine Lee[1], Kevin Croft[5], Trevor Mori[5], Barbara Scheiber-Mojdehkar[6], Brigitte Sturm[6], Monika Praschbergerg[6], Adam Vogel[3], Gary Rance[3], Sarah Stephenson[3], Joseph Sarsero[1], Creina Stockley[7], Jetty Lee[8], Andrew Churchyard[4], Marguerite Evans-Galea[1], Monique Ryan[1, 2, 3], Paul Lockhart[1, 3], Louise Corben[1, 4], Martin Delatycki[1, 3, 9]

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**Introduction:** Friedreich ataxia (FRDA) is due to a triplet repeat expansion in the FXN gene, resulting in deficiency of the mitochondrial protein frataxin. Resveratrol is a plant-derived polyphenol. It was identified to increase frataxin expression in cellular and mouse models of FRDA, and has anti-oxidant properties.

**Methods:** This trial evaluated the effect of two different doses of resveratrol on lymphocyte frataxin levels over a 12-week period in individuals with FRDA. Secondary aims evaluated the effect on FXN mRNA, oxidative stress markers and clinical measures of disease severity. Safety and tolerability were studied.

**Results:** 24 participants completed the study; 12 received low-dose resveratrol (1g daily) and 12 high-dose resveratrol (5g daily). Lymphocyte frataxin levels did not change in either dosage group [low dose group change: 0.08 pg/µg protein (95% CI -0.05, 0.21, p=0.21); high dose group change: 0.03 pg/µg protein (95% CI -0.10, 0.15, p=0.62)]. Improvement in ataxia was evident in the high-dose group (change in International Cooperative Ataxia Rating Scale, ICARS -1.9 points, 95% CI -3.1, -0.8, p=0.004) but not the low-dose group (change in ICARS -0.3 points, 95% CI -3.2, 2.6, p=0.80). Significant improvements in hearing and speech were demonstrated in the high-dose group. A significant decrease in the oxidative stress marker plasma F2-isoprostanes occurred in the high-dose group. No serious adverse events were recorded. Gastrointestinal side effects were a common, dose-related adverse event.

**Conclusions:** This trial provides promising evidence for high-dose resveratrol as a potential disease-modifying therapy for FRDA. A placebo-controlled trial is required to assess whether resveratrol is clinically beneficial in FRDA.

**P107**

## Lithium trial in Machado Joseph disease: subgroup analysis and responsiveness of rating scales

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**Objectives**: To further analyze the data of the lithium carbonate trial (Saute et al, 2014) in Machado-Joseph disease (MJD/SCA3), searching for treatment response modifiers, performing subgroup analysis, and determining additional metric properties of spinocerebellar ataxias (SCA) scales.

**Methods**: 62 MJD/SCA3 patients had been randomly assigned (1:1) for the double-blind, placebo-controlled trial. We performed additional analysis with the subscores of the Neurological Examination Score for the Assessment of Spinocerebellar Ataxia (NESSCA) and the Scale for the Assessment and Rating of Ataxia (SARA) and with the subgroup of patients with independent gait. Potential interactions of clinical/molecular findings with treatment response; minimally important differences (MID); and sample size estimations (with placebo data) of NESSCA, SARA, Spinocerebellar Ataxia Functional-Index (SCAFI) and Composite-Cerebellar Functional-Score (CCFS) were evaluated.

**Results**: Cerebellar NESSCA differed between groups over the whole 48 weeks ($p<0.001$), favoring lithium. Gait ataxia severity interacted significantly with treatment response on SCAFI ($p=0.010$), with a minor progression with lithium therapy for patients with independent walking. NESSCA ($p=0.010$) and SCAFI ($p=0.015$) differed between groups in the subgroup of patients able to perform the 8-meters walking-time, favoring lithium. Estimated sample sizes with the evaluated scales were provided for future trials.

**Conclusions**: Lithium efficacy on cerebellar NESSCA and on SCAFI and CCFS in the primary analysis suggests lithium efficacy on cerebellar features of MJD/SCA3. The interaction of disease severity with treatment response on SCAFI and NESSCA indicates that early stages patients should be preferentially recruited. We provide relevant data for planning future clinical trials, especially with lithium, in MJD/SCA3.

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### Systematic review of treatment options for dysarthria in hereditary ataxia syndromes

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**Background**: Hereditary ataxia syndromes typically result in significant speech impairment, a symptom thought to be responsive to treatment. People with ataxia often present with slower and unclear speech, dramatically restricting their personal, social and employment opportunities.

**Objective**: To assess the effects of interventions for speech disorder in adults and children with Friedreich ataxia and other hereditary ataxias.

**Methods**: Cochrane systematic review of evidence supporting speech rehabilitation in hereditary ataxia syndromes.

**Results**: Fourteen clinical trials, involving 721 participants, met the criteria for inclusion in the review. Thirteen studies compared a pharmaceutical treatment with placebo (or a low dose of the intervention), in heterogenous groups of degenerative cerebellar ataxias and the 14th compared a mixed physiotherapy and occupational therapy treatment to no treatment. The duration of treatment was between two weeks and two years. Ten different compounds were tested: L-hydroxytryptophan (L-5HT) (two studies), thyrotropin- releasing hormone (TRH) (two studies), varenicline, riluzole, idebenone (two studies), betamethasone, coenzyme Q10 with vitamin E, buspirone, αtocopheryl quinone, and erythropoietin. No studies utilised traditional speech therapies.

Five studies reported statistically significant improvement on an overall disease rating scale in which a speech subscale was included. Only three of those studies provided specific data on speech performance; all were comparisons with placebo. Improvements in overall disease severity were observed with α-tocopheryl quinone; however, no significant changes were found on the speech subscale in a group of individuals with Friedreich ataxia. A statistically significant improvement in speech according to a speech disorders subscale was observed with betamethasone. Riluzole was also found to have a statistically significant effect on speech in a group of participants with mixed hereditary, sporadic and unknown origin ataxias. It is difficult to say whether these improvements in speech might make a meaningful difference to patients. No significant differences were observed between treatment and placebo in any other pharmaceutical study.

**Conclusions**: There is insufficient and low or very low quality evidence from either RCTs or observational studies to determine the effectiveness of any treatment for speech disorder in any of the hereditary ataxia syndromes.

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## Riluzole in hereditary cerebellar ataxia: a randomized double-blind placebo controlled trial

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Small conductance calcium-activated potassium (SK) channels openers such as the drug riluzole, may reduce neuronal hyperexcitability and thereby be useful in the therapy of cerebellar ataxia. On this base we performed a pilot study in patients with chronic cerebellar ataxia (irrespective of etiology), investigating safety and efficacy of riluzole or placebo administration for 8 weeks. During the study no major side effects was reported and the results wre encouraging (Ristori et al. Neurology 2010).

The present study was aimed at verifying the safety and efficacy of riluzole administration for a longer period, in a larger sample size of patients, with more stringent diagnostic criteria (hereditary cerebellar ataxia). In a multicentre study, sixty patients were enrolled between May 2010 and February 2013, in a double-blind, placebo-controlled trial. By central randomisation, patients took 50 mg of riluzole (Rilutek®) or placebo twice daily for 12 months. Treatment effects was assessed by comparing the Scale for the Assessment and Rating of Ataxia (SARA) before treatment and during therapy at months 3 (time point suggestive of 'symptomatic' effect) and 12 (time point suggestive of effect on disease progression). Occurrence of adverse effects was also compared between the two arms.

Data were expressed as mean (SD) for continuous variables and as proportions for categorical variables. Comparisons between the two groups were assessed using the t test for unpaired data and chi-square for categorical data. P values < 0.05 were considered significant. All the analysis were performed using the SPSS statistical package (version 17).

Riluzole arm performed significantly better at both time points: the mean changes of SARA score compared to baseline was – 1.16 ±1.84 vs 0.54 ± 2.37 at month 3, and – 1.18 ± 2.28 vs 1.72 ± 2.84 at month 12 (p = 0.007 and 0.0002 at t Student's test). The proportion of patients stable or improved at SARA score was significantly higher in the riluzole group at months 3 [92% vs 64%; p = 0.01 at c2 test; relative risk 0.22 (0.05-0.93)] and 12 [0.84% vs 48%; p = 0.007; relative risk 0.31 (0.12-0.81)]. No major adverse event occurred during the trial. Two patients showed an increase in liver enzymes (<1.5 times above normal limits).

This study confirms beneficial symptomatic effect of riluzole in patients with cerebellar ataxia, while the adverse events were within the known safety profile of the drug. The long-term action (at least 12 months), that was observed in patients with hereditary diseases, suggests to possibly consider riluzole as front line therapy in incurable forms of cerebellar ataxia.

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## Risk of heart failure and death among patients with Friedreich ataxia admitted for non-cardiac etiologies

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**Background**: Friedreich Ataxia (FA) is an autosomal recessive neurosensory disorder with an associated cardiomyopathy. As a rare disorder, little is known about the effect of heart failure (HF) on inpatient hospitalizations in FA patients. We hypothesized that a diagnosis of HF in FA is associated with higher mortality and hospital charges than those without HF.

**Methods**: A retrospective analysis of the Healthcare Cost and Utilization Project Kids' Inpatient Database (KID) and Nationwide Inpatient Sample (NIS) was performed for pediatric and adult hospitalizations during the years 2000, 2003, 2006, and 2009. Subjects with the diagnosis of FA were identified using ICD-9 codes. Primary diagnoses were grouped into 18 categories and sorted according to whether HF or death occurred during each hospitalization.

**Results**: There were 1094 FA hospitalizations identified during the years studied, with a mean age of 39 ± 19 years. Most patients with FA are hospitalized for non-cardiac etiologies (cardiac 188, non-cardiac 906). Most common non-cardiac reasons for hospitalization are infectious (193), neurologic (127), GI (116), and musculoskeletal (107). 84 (9%) of admissions with a non-cardiac primary diagnosis were complicated by HF; patients with and without HF were of similar age. HF patients with a non-cardiac primary diagnosis had similar length of stay (7.8 vs 6.5 days) and hospital charges ($37971 vs $29897), but greater mortality (14% vs 4%, OR 3.96 95%CI 1.96-8.01, $p<0.001$).

**Conclusions**: 9% of non-cardiac admissions in FA patients are complicated by heart failure. Heart failure is associated with 4 fold increase in the risk of death in these patients. Further study is needed to identify effective means of cardiac surveillance and treatment in this high risk population.

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## Prevalence of autoantibodies and efficacy of immunotherapy in autoimmune cerebellar ataxia: a study in Japan

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Autoimmune cerebellar ataxias, including anti-glutamic acid decarboxylase (GAD) antibody-positive cerebellar ataxia, gluten ataxia, and Hashimoto's encephalopathy, were recently reported to be treatable. However, the proportion of patients with cortical cerebellar atrophy of unknown etiology with autoimmune-associated cerebellar ataxia and the actual effectiveness of immunotherapy in these diseases remain unknown. In the present study in Japan, 32 of 59 (54%) patients were positive for anti-GAD antibody, anti-gliadin antibody, deamidated gliadin peptide antibody, or anti-thyroid antibody. Seven of the 12 anti-gliadin or deamidated gliadin peptide antibody-positive patients, two of the three anti-GAD antibody-positive patients, and three of the six solely anti-thyroid antibody-positive patients responded well to immunotherapy, indicating that 12 of the 21 patients (57%) with autoantibody-positive cerebellar ataxia responded well. Thus, some patients with cerebellar ataxia have autoimmune conditions, and diagnosing autoimmune cerebellar ataxia is an important component in the care of patients with this disease entity.

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## Stabilized polyunsaturated fatty acid drug recovers mitochondrial function in multiple Friedreich ataxia models: a planned Phase 1b/2a trial in patients

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Polyunsaturated fatty acids (PUFAs) are susceptible to an accelerating damage cascade from an autocatalytic, free radical chain reaction. Damaged lipid end products (e.g. 4-hydroxy-nonenal and others) from this process have been associated with mitochondrial dysfunction and a host of age-related degenerative diseases, including Friedreich Ataxia (FRDA). Cells in multiple models of FRDA, when treated with a stabilized lipid mimetic of the normal dietary PUFAs in mitochondrial membranes, show stunning reversal of lipid peroxidation damage, increased cell viability, and improved mitochondrial function. The mechanism of action of the drug, a stabilized form of the essential fat linoleic acid, is believed to be down-regulation of PUFA autoxidation initiated by hydrogen abstraction from susceptible, bis-allylic sites of mitochondrial membrane PUFAs. Replacement of the bis-allylic hydrogen atoms with deuterium atoms (D-PUFAs) arrests PUFA autoxidation in vitro and in vivo due to the kinetic isotope effect. Unlike antioxidants, which are typically consumed as they quench lipid peroxidaiton products, D-PUFAs are not used up in the process of inhibiting lipid peroxidation, and don't suffer from the distribution and diffusion limitations of antioxidant approaches.

Surprisingly, cells from yeast, murine, and human (primary FRDA patient cells) treated with a mixture of approximately only 20% isotope-reinforced D-PUFA in a background of normal PUFAs are fully protected from lipid autoxidation-mediated cell killing. The findings also show mitigation of mitochondrial dysfunction and increased cell viability. As a minor perturbation on naturally occurring GRAS fats, D-PUFA drugs enjoy all the active transport in an out of tissues and mitochondria that evolved over decades to esnure critical PUFA molecules were replaced when damaged, and were granted an accelerated pathway into human testing by the US FDA. A trial in the orphan neurodegenerative disease, Friedreich Ataxia, is planned. Orally fed rodent models in other degenerative diseases and PK studies of the drug confirm efficacy in difficult to reach brain and retina tissues, and IND-enabling toxicity studies showed no signs of drug related adverse findings in any parameter tested.

The planned Phase 1b/2a trial in 33 patients dosed for 6 months is expected to start in early 2015, will include an ascending dose safety study, and will measure FARS and multiple other FRDA disease readouts.