In this last issue of EFACTS NEWS, we would like to present another update on the status of the EFACTS patient registry which has begun to see patients at their third annual follow-up visits.

Since the recruitment of a “core sample” was completed in the spring of 2013, baseline data analyses have been underway and baseline results will be published shortly. Basic science projects have provided a lot of new important knowledge and short summaries of findings of the past year can be found in this newsletter.

Finally, we would like to report on the progress of the nicotinamide trial, present results from the completed nicotinamide dose-escalation trial, and give you an outlook on the future of the EFACTS consortium and registry.
The 604 patients who were enrolled by 30th April 2013 were considered the “core sample” of EFACCTS and baseline analyses of collected patient data were based on this group of patients. Results from these analyses will be published shortly. However, the Consortium expects that for future treatment trials, a large number of patients will need to be identified to obtain conclusive results on the effectiveness of tested treatments. Therefore, the registry remains open for new patients!

Enrolment and retention
In addition to the 604 patients of the “core sample”, who are the largest group of Friedreich’s ataxia patients ever followed for a minimum of 2 years to collect natural history data, the registry has accepted 37 new patients since spring 2013. 1-year follow-up assessments on 496 patients of the “core sample” were concluded in May 2014 and analyses of collected data have begun. By mid-October 2014 341 “core sample” patients had returned for their 2-year follow-up assessment and 47 patients for their third annual follow-up assessment.

“Core sample” enrolment and retention by centre

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Participant characteristics at baseline

<table>
<thead>
<tr>
<th>Characteristic</th>
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<tr>
<td>Male (%) / female (%)</td>
<td>299 (47) / 342 (53)</td>
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<tr>
<td>Mean age in years at (range)</td>
<td>34 (6-76)</td>
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<tr>
<td>No. of children (&lt;18 years)</td>
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</table>

THANK YOU FOR YOUR INVESTMENT IN THE REGISTRY, HELPING US FIND A TREATMENT FOR FRIEDREICH’S ATAXIA!
Epigenetic and neurological effects and safety of high-dose nicotinamide in patients with Friedreich's ataxia: an exploratory, open-label, dose-escalation study.


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. Preclinical studies have shown that the histone deacetylase inhibitor nicotinamide (vitamin B3) can remodel the pathological heterochromatin and upregulate expression of FXN. 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. This study is registered with ClinicalTrials.gov, number NCT01589809. 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=0.0004). 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<0.0001) upregulation of frataxin expression, which was accompanied by a reduction in heterochromatin modifications at the FXN locus. In the short period of time, 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.
Deferiprone in Friedreich's Ataxia: A six-month randomized controlled trial.


We conducted a 6-month randomized, double-blind, placebo-controlled study to assess safety, tolerability, and efficacy of deferiprone in Friedreich's ataxia. Seventy-two patients were treated with deferiprone 20, 40, or 60 mg/kg/day or placebo, divided into two daily doses. Safety was the primary objective, secondary objectives included standardized neurological assessments (FARS, ICARS, 9HPT, T25FW, LCLA), general functional status (ADL), and cardiac assessments. Deferiprone was well tolerated at 20 mg/kg/day, whereas more adverse events occurred in the 40 mg/kg/day than in the placebo group. The 60mg/kg/day dose was discontinued due to worsening of ataxia in two patients. One patient on deferiprone 20 mg/kg/day experienced reversible neutropenia, but none developed agranulocytosis. Deferiprone-treated patients receiving 20 or 40 mg/kg/day showed a decline in the left ventricular mass index, compared to an increase in the placebo-treated patients. Patients receiving 20 mg/kg/day of deferiprone had no significant change in FARS, similar to the placebo-treated patients, while those receiving 40 mg/kg/day had worsening in FARS and ICARS scores. The lack of deterioration in the placebo arm impaired the ability to detect any potential protective effect of deferiprone. However, subgroup analyses in patients with less severe disease suggested a benefit of deferiprone 20 mg/kg/day on ICARS, FARS, kinetic function and 9HPT. This study demonstrated an acceptable safety profile of deferiprone at 20 mg/kg/day for the treatment of patients with FRDA. Subgroup analyses raise the possibility that, in patients with less severe disease, deferiprone 20 mg/kg/day may reduce disease progression, while higher doses appear to worsen ataxia.

MutLα heterodimers modify the molecular phenotype of Friedreich ataxia.


LAY ABSTRACT

Previous studies of FRDA cell and mouse models have revealed a role for certain proteins that usually repair damaged DNA, called mismatch repair proteins, in GAA repeat instability: MSH2, MSH3 and MSH6 promote GAA repeat expansions, while PMS2 inhibits GAA repeat expansions. These effects may play important roles in FRDA disease progression. By studying FRDA mouse models, we have now investigated the potential role of another mismatch repair protein, MLH1, in GAA repeat instability. We find that MLH1 promotes GAA repeat expansions. However, we also find that loss of MLH1 and PMS2 can also reduce frataxin expression levels. Therefore, both PMS2 and MLH1 have now been shown to modify the molecular phenotype of FRDA. We propose that upregulation of MLH1 or PMS2 could be potential FRDA therapeutic approaches to increase frataxin expression.
Prevention and reversal of severe mitochondrial cardiomyopathy by gene therapy in a mouse model of Friedreich's ataxia.


Cardiac failure is the most common cause of mortality in Friedreich's ataxia (FRDA), a mitochondrial disease characterized by neurodegeneration, hypertrophic cardiomyopathy and diabetes. FRDA is caused by reduced levels of frataxin (FXN), an essential mitochondrial protein involved in the biosynthesis of iron-sulfur (Fe-S) clusters. Impaired mitochondrial oxidative phosphorylation, bioenergetics imbalance, deficit of Fe-S cluster enzymes and mitochondrial iron overload occur in the myocardium of individuals with FRDA. No treatment exists as yet for FRDA cardiomyopathy. A conditional mouse model with complete frataxin deletion in cardiac and skeletal muscle (Mck-Cre-Fxn(L3/L-) mice) recapitulates most features of FRDA cardiomyopathy, albeit with a more rapid and severe course. Here we show that adeno-associated virus rh10 vector expressing human FXN injected intravenously in these mice fully prevented the onset of cardiac disease. Moreover, later administration of the frataxin-expressing vector, after the onset of heart failure, was able to completely reverse the cardiomyopathy of these mice at the functional, cellular and molecular levels within a few days. Our results demonstrate that cardiomyocytes with severe energy failure and ultrastructure disorganization can be rapidly rescued and remodeled by gene therapy and establish the preclinical proof of concept for the potential of gene therapy in treating FRDA cardiomyopathy.

hFXN protein expression assessed by immunofluorescence in heart from 35-week-old treated Mck mice using antibody to FXN.
Mitochondrial dysfunction induced by frataxin deficiency is associated with cellular senescence and abnormal calcium metabolism.


LAY ABSTRACT
Frataxin deficiency is the cause of Friedreich ataxia (FRDA), a neurodegenerative and systemic disorder that has the dorsal root ganglia (DRG) as a major target neural tissue. In this manuscript we are addressing new knowledge on the effect of frataxin depletion on mitochondria and cells, and how modifications in the mitochondrion may affect different cellular processes. We have investigated the mitochondrial and cellular consequences of frataxin deficiency in a neuron-like model based on FXN gene silencing in the human neuroblastoma cell line SH-SY5Y. Frataxin silencing provoked slow cell growth associated with cellular senescence. Cellular senescence should be considered as a contributing factor for the incomplete neurodevelopment as suggested by necropsy studies. In an attempt to understand such cellular senescence and its relationship with the disease neuropathology, we focused not only on the characterization of bioenergetics and redox status of the cell model but also on other mitochondrial functions that have been associated with neurodegeneration that have not received enough attention in FRDA pathogenesis (mitochondrial dynamics and calcium homeostasis). We show that the reduction of frataxin induces mitochondrial dysfunction due to a bioenergetic deficit and reduced Ca2+ uptake capacity of the mitochondria that were associated with both oxidative and endoplasmic reticulum (ER) stresses. The ER stress is a cellular response from conditions that interfere with the correct function of the ER, such as changes of the Ca2+ homeostasis. In addition, the depletion of frataxin does not cause cell death but increased autophagy, an intracellular degradation system, which may have a protective effect against cellular insults such as oxidative stress. However, under conditions of prolonged stress signals, ER stress induces apoptosis. We propose that the manipulation of mitochondrial Ca2+ homeostasis, ER stress, and cellular senescence should be explored as a potential therapeutic strategy for the treatment of FRDA patients.

Quantification of the mitochondrial dynamic process in SH-SY5Y (human neuroblastoma cells). The representative mitochondrial network shows increased fusion morphology in frataxin-deficient cells.

© 2014 Bolinches-Amorós, Mollá, Pla-Martín, Palau and González-Cabo.
For a more comprehensive understanding of Friedreich’s ataxia, past research findings and opinions on needed future research studies please also see these review articles, which have been published by EFACS investigators in the past year:

**Epigenetic-based therapies for Friedreich ataxia.**
Sandi C, Sandi M, Anjomani Virmouni S, Al-Mahdawi S, Pook MA.

Friedreich ataxia (FRDA) is a lethal autosomal recessive neurodegenerative disorder caused primarily by a homozygous GAA repeat expansion mutation within the first intron of the FXN gene, leading to inhibition of FXN transcription and thus reduced frataxin protein expression. Recent studies have shown that epigenetic marks, comprising chemical modifications of DNA and histones, are associated with FXN gene silencing. Such epigenetic marks can be reversed, making them suitable targets for epigenetic-based therapy. Furthermore, since FRDA is caused by insufficient, but functional, frataxin protein, epigenetic-based transcriptional re-activation of the FXN gene is an attractive therapeutic option. In this review we summarize our current understanding of the epigenetic basis of FXN gene silencing and we discuss current epigenetic-based FRDA therapeutic strategies.

**Dysregulation of cellular iron metabolism in Friedreich ataxia: from primary iron-sulfur cluster deficit to mitochondrial iron accumulation.**
Martelli A, Puccio H.

Friedreich ataxia (FRDA) is the most common recessive ataxia in the Caucasian population and is characterized by a mixed spinocerebellar and sensory ataxia frequently associating cardiomyopathy. The disease results from decreased expression of the FXN gene coding for the mitochondrial protein frataxin. Early histological and biochemical study of the pathophysiology in patients’ samples revealed that dysregulation of iron metabolism is a key feature of the disease, mainly characterized by mitochondrial iron accumulation and by decreased activity of iron-sulfur cluster enzymes. In the recent past years, considerable progress in understanding the function of frataxin has been provided through cellular and biochemical approaches, pointing to the primary role of frataxin in iron-sulfur cluster biogenesis. However, why and how the impact of frataxin deficiency on this essential biosynthetic pathway leads to mitochondrial iron accumulation is still poorly understood. Herein, we review data on both the primary function of frataxin and the nature of the iron metabolism dysregulation in FRDA. To date, the pathophysiological implication of the mitochondrial iron overload in FRDA remains to be clarified.

**Chronochemistry in neurodegeneration.**
Pastore A, Adinolfi S.

The problem of distinguishing causes from effects is not a trivial one, as illustrated by the science fiction writer Isaac Asimov in a novel dedicated to an imaginary compound with surprising “chronochemistry” properties. The problem is particularly important when trying to establish the etiology of diseases. Here, we discuss how the problem reflects on our understanding of disease using two specific examples: Alzheimer’s disease (AD) and Friedreich’s ataxia (FRDA). We show how the fibrillar aggregates observed in AD were first denied any interest, then to assume a central focus, and to finally recess to be considered the dead-end point of the aggregation pathway. This current view is that the soluble aggregates formed along the aggregation pathway rather than the mature amyloid fiber are the causes of disease. Similarly, we illustrate how the identification of causes and effects have been important in the study of FRDA. This disease has alternatively been considered as the consequence of oxidative stress, iron precipitation or reduction of iron-sulfur cluster protein context. We illustrate how new tools have recently been established which allow us to follow the development of the disease. We hope that this review may inspire similar studies in other scientific disciplines.
**Epigenetic therapy for Friedreich's ataxia.**


Objective: To investigate whether a histone deacetylase inhibitor (HDACi) would be effective in an in vitro model for the neurodegenerative disease Friedreich's ataxia (FRDA) and to evaluate safety and surrogate markers of efficacy in a phase I clinical trial in patients. Methods: We used a human FRDA neuronal cell model, derived from patient induced pluripotent stem cells, to determine the efficacy of a 2-aminobenzamide HDACi (109) as a modulator of FXN gene expression and chromatin histone modifications. FRDA patients were dosed in four cohorts, ranging from 30 mg/day to 240 mg/day of the formulated drug product of HDACi 109, RG2833. Patients were monitored for adverse effects as well as for increases in FXN mRNA, frataxin protein, and chromatin modification in blood cells. Results: In the neuronal cell model, HDACi 109/RG2833 increases FXN mRNA levels and frataxin protein, with concomitant changes in the epigenetic state of the gene. Chromatin signatures indicate that histone H3 lysine 9 is a key residue for gene silencing through methylation and reactivation through acetylation, mediated by the HDACi. Drug treatment in FRDA patients demonstrated increases in FXN mRNA and H3 lysine 9 acetylation in peripheral blood mononuclear cells. No safety issues were encountered. Interpretation: Drug exposure inducing epigenetic changes in neurons in vitro is comparable to the exposure required in patients to see epigenetic changes in circulating lymphoid cells and increases in gene expression. These findings provide a proof of concept for the development of an epigenetic therapy for this fatal neurological disease. © 2014 American Neurological Association.

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**‘Switching’ the Frataxin gene back ‘on’ in Friedreich’s ataxia**

Prof. Richard Festenstein, Imperial College, London, UK

The Festenstein lab previously demonstrated that GAA-triplet repeats can induce gene silencing in a manner previously described for the epigenetic phenomenon of position effect variegation (PEV) (Saveliev et al., 2003) which has been instrumental in the development of the notion of a histone or epigenetic code (Jenuwein and Allis, 2001). This implicated chromatin modifiers as disease modifiers in Friedreich's ataxia (Chan et al., 2013). In the last newsletter it was reported that an exploratory clinical study was underway to determine whether the histone deacetylase inhibitor, nicotinamide, could upregulate the frataxin gene in patients with Friedreich's ataxia. This study revealed upregulation of frataxin in patients using large doses of nicotinamide which were generally well tolerated (Libri et al., 2014). The results support further investigation of the safety and clinical efficacy in a larger randomised controlled clinical trial which is being planned. If frataxin can be safely upregulated in a sustained manner it would be predicted that the treatment may prevent further decline in this otherwise relentlessly progressive condition. Friedreich’s ataxia could be viewed as a prototypic position-effect disease making it possible that other diseases caused by a similar mechanism would be amenable to similar approach.

References:


The Future of the Registry
Prof. Jörg B. Schulz, University Hospital RWTH Aachen, Germany

Funding of the EFACS registry by the European Commission will come to an end by April 2015. By then, we will have a complete 2 year follow-up of all patients which were retained in the database and a 3rd year investigation by more than 50% of the patients. We have just applied for new funding through the European Commission’s framework program Horizon 2020. If successful, this funding will financially secure the patient registry and yearly follow-up investigations for four years. We consider this to be of importance for successfully planning, designing, and running future clinical interventional trials, testing e.g. gene therapeutic approaches, HDAC inhibitors or other approaches for their safety and effectiveness. A decision on our grant application will not be available before summer or fall 2015. The registry will therefore need to be run without funding starting in May 2015. Meanwhile, we would welcome short-term financial support from patient associations and the industry and we would like to strongly encourage patients and investigators to continue yearly follow-up assessments (without financial compensation until additional funding is secured), thereby keeping the registry and its value alive.

Meetings & Dates

Neuroscience 2014
15th - 19th November 2014
Washington, DC, USA

International Ataxia Research Conference
25th – 28th March 2015
Beaumont Estate, Windsor, UK

International Conference on Rare Diseases and Orphan Drugs
12th – 17th October 2015
Mexico City, Mexico

28th February 2015:
Rare Disease Day
25th September 2015:
International Ataxia Awareness Day

Attendants of the 4th annual meeting of the EFACS consortium, 27th – 28th June 2014,
Centro de Investigación Príncipe Felipe, Valencia, Spain
Photo courtesy of Dr. Michael H. Parkinson (m.parkinson@ucl.ac.uk).