EFACTS
Research For a Better Future

EUROPEAN FRIEDREICH'S ATAXIA
CONSORTIUM FOR TRANSLATIONAL STUDIES

PROJECT FINAL REPORT

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Executive summary

EFACTS (the European Friedreich's Ataxia Consortium for Translational Studies) assembles a body of expertise to adopt a translational research strategy for the rare autosomal recessive neurological disease, Friedreich ataxia (FRDA).
FRDA is a severely debilitating disease that leads to loss of the ability to walk and dependency for all activities. Some patients have cardiomyopathy that can cause premature death, visual and auditory loss, kyphoscoliosis, pes cavus, and diabetes. Onset is usually in childhood, but it may vary from infancy to adulthood. FRDA involves child health and ageing aspects.
FRDA affected individuals and clinical specialists are dispersed. This is a hindrance for patients to receive the care they need, and for clinicians and researchers to make progress. EFACTS has been created to move past this limitation.
The consortium, led by Professor Massimo Pandolfo (Neurologist, Université Libre de Bruxelles, Brussels, Belguim) acting as coordinator, possesses expertise ranging from clinical neurology, biochemistry, structural biology, systems biology, genetics and epigenetics. These 14 clinical and basic investigators are undisputed leaders in FRDA research and have provided major contributions to the current knowledge of this disease. EFACTS strongly believes that, 12 years after European researchers discovered the FRDA gene, frataxin, when new treatments for FRDA are being developed, the time is ripe to invest in FRDA research in a concerted Europe-wide fashion.
EFACTS gathered the critical mass of researchers and clinicians to exploit the patient base, research reagents and knowledge for progress. This came when IT can act as a crucial support for collaborative work in collecting patient data and material, making it available to leading researchers for advanced analysis, research and drug development.
To this end, this project has drawn up and executed a joint programme of research with the following scientific and technological objectives, providing a model for translational European research targeted to a specific rare disease, which can ultimately improve the patients' health and quality of life.

1. Comprehensively populate a European FRDA database, linked to a bio bank
2. Define a panel of clinical assessment tools that can be used to define outcomes for clinical trials and identify the multiple health problems that occur during the course of FRDA disease progression
3. Build on the knowledge base of frataxin structure and function
4. Build on the knowledge base of frataxin-related cellular homeostasis and the pathogenic cascade
5. Build on the knowledge base of epigenetic mechanisms regulating frataxin silencing
6. Develop new and improved cellular and animal models for the study of FRDA
7. Identify novel FRDA biomarkers
8. Identify genetic modifiers of FRDA disease
9. Develop novel therapeutic strategies for treating FRDA

Summary description of the project context and the main objectives.

Concept

The overall concept developed by EFACTS (the European Friedreich Ataxia Consortium for Translational Studies) is to assemble a body of expertise that can adopt a fully translational research strategy applied to the study and treatment of Friedreich ataxia (FRDA), a rare monogenic neurological disease. FRDA is a severely debilitating disease that leads to loss of the ability to walk and dependency for all activities. Some patients have cardiomyopathy that can cause premature death, visual and auditory loss, kyphoscoliosis, pes cavus, and diabetes. Onset is usually in childhood, but it may vary from infancy to adulthood.

FRDA affected individuals and clinical specialists are dispersed. This is a hindrance for patients to receive the care they need, and for clinicians and researchers to make progress. EFACTS has been created to move past this limitation. The consortium possesses expertise ranging from clinical neurology, biochemistry, structural biology, systems biology, genetics and epigenetics. These clinical and basic investigators are undisputed leaders in FRDA research and have provided major contributions to the current knowledge of this disease.

Objectives

EFACTS overall goal is to progress our understanding of FRDA through basic research and harness this for identifying novel disease-specific biomarkers and drug therapeutics. This will be coupled to improving the methods of clinical assessment and diagnosis, and the implementation of the first pan-European FRDA database registry, linked to bio banks of patient material.

The totality of objectives provide a model for translational European research targeted to a specific disease, which can ultimately improve the patients' health and quality of life.

To this end, this project has drawn up a joint programme of research with the following scientific and technological objectives:

1. Comprehensively populate a European FRDA database, linked to a bio bank
2. Define a panel of clinical assessment tools that can be used to define outcomes for clinical trials and identify the multiple health problems that occur during the course of FRDA disease progression
3. Build on the knowledge base of frataxin structure and function
4. Build on the knowledge base of frataxin-related cellular homeostasis and the pathogenic cascade
5. Build on the knowledge base of epigenetic mechanisms regulating frataxin silencing

6. Develop new and improved cellular and animal models for the study of FRDA
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9. Develop novel therapeutic strategies for treating FRDA

Objectives 1-2

An IT-based patient registry, fulfilling all data protection requirements, has been implemented. Up to 30th April 2015, 604 patients were enrolled, forming a "core" cohort with a planned minimal follow-up of two years. So far, close to 83% have undergone their first annual follow-up assessment, and 71% already came back for the second one. Baseline data of the "core" cohort have been analysed and published in Lancet Neurology. A biological repository has been established, where samples from EFACTS patients are stored for analyses. It currently contains 559 baseline and 461 1year follow-up samples.

Objectives 3-4

EFACTS beneficiaries could establish that frataxin, by controlling both iron entry and sulfide production, is essential to properly assemble and protect the Fe-S cluster during the initial stage of biogenesis. They confirmed that *in vitro* human frataxin functions as an enzyme activator while, under the same conditions, the *E. coli* homologue CyaY is an inhibitor of Fe-S cluster biosynthesis. Conversely, the previously postulated participation of frataxin in heme biosynthesis could not be confirmed.

Investigations on the pathogenic cascade in FRDA included analyses of mitochondrial function, iron metabolism, ROS production, apoptosis and signalling pathways in human and mouse cells, and a proteomic study in a fly model.

Objective 5

Epigenotype analysis has identified key histone and DNA modifications associated with *FXN* gene silencing triggered by expanded GAA repeats. A novel methodology enabling the localisation of the *FXN* gene and its activity to specific regions in the nucleus in living cells has been established – this is important for understanding the mechanisms underlying the silencing of the gene in FRDA and provides an excellent system for studying the ability of novel therapies to relocate genes from silencing compartments to active regions in the nucleus. Additionally, a novel rapid technique for assessing DNA methylation, allowing confirmation of specific DNA methylation sites in FRDA, has been developed.

Objective 6

Construction of a complete human transgene with large GAA repeat expansion flanked by loxP sites and with a mutated pausing site is under way. It will be used to generate a mouse model closely replicating the genetic and epigenetic features of the human disease. The development of cellular models has focused

on the use of induced pluripotent stem cells (iPSCs). EFACTS published data show for the first time that iPSCs and their neuronal and cardiac derivatives allow to study mitochondrial damages and GAA expansion instability in FRDA.

Objective 7

Collection of biological samples for biomarker studies is ongoing. A first study on gene expression profiles in FRDA PBMCs established a core biomarker set and evaluated how it is affected by compounds that restore frataxin expression (HDAC inhibitors). A subsequent study showed that *ex vivo* nicotinamide treatment of PBMCs normalizes about 67% of these biomarkers, confirming that they can be used to monitor response to pharmacologically induced increase of frataxin levels.

Objective 8

The identification of genes that modify the frataxin deficiency phenotype or affect frataxin silencing by expanded GAA repeats has been pursued using the powerful genetic approaches that are possible in Drosophila. New lines carrying expanded GAA repeats were screened for modifiers of GAA repeat-induced silencing and for modifiers of repeat stability. Along with bioinformatics analysis of biomarkers obtained from all relevant beneficiaries and from published data, these studies will yield important clues about FXN gene silencing and the downstream effects of FXN silencing.

Objective 9

EFACTS has focused on potential therapeutics to increase FXN expression, with these main achievements so far:

- The first-in-human study of diphenylamide HDAC inhibitors has shown that these molecules can safely up-regulate frataxin expression in FRDA patients, at least in peripheral tissues.
- The identification of a new compound, C5 that could be a potential therapeutic for FRDA.
- Three compounds (IFN-γ, NAM, and a proteasome inhibitor) have been tested in the YG8 mouse model of FRDA with encouraging results.
- The proof-of-concept demonstration in an animal model that AAVrh10-based gene therapy can restore frataxin expression in the hearth and reverse FRDA cardiomyopathy even when already symptomatic.
- Phase 1 clinical testing of nicotinamide, an HDAC class 3 inhibitor, with positive results in terms of safety and *FXN* induction.
- Phase 1 clinical testing of a 2-aminobenzamide HDAC inhibitor, with positive results in terms of safety and *FXN* induction.