



A Collaborative Approach

FARA funds progress – and our vision is a world without the debilitating effects of FA. FARA is a worldwide leader in growing and supporting the FA research community through our robust research grant program, patient registry and investment in clinical research infrastructure, international conferences and advocacy and partnership with government organizations and pharmaceutical companies. We have invested more than \$25 million, through a competitive grant program, and leveraged about the same amount from government agencies, other non-profit organizations and academic partners to advance the basic and discovery science. FARA is a collaborative organization, which is proud to work closely with sister organizations. Our collaborative approach has allowed FARA to:

- Advance the understanding of the genetic etiology of FA
- Determine the cellular dysfunction and pathways impacted in FA
- Discover and validate animal and cellular models
- Validate clinical outcome measures and biomarkers

FARA can bring significant resources and infrastructure to a therapeutic development program. FARA is committed to helping cut preclinical and clinical development time to a minimum and getting products to market faster and at a lower cost. Our collaborations and meetings with international partners give us global reach, with access to expertise, patients and clinicians around the world. Horizon Pharma plc had the following to say regarding FARA's collaborate approach:

“The FARA team has been most helpful in introducing Horizon Pharma to key stakeholders and collaborators in the FA research and patient communities, ensuring that we are working with the best people.

We will be utilizing both clinical sites and data management services in the Collaborative Clinical Research Network (CCRN) in FA for our upcoming ACTIMMUNE Phase 3 study. FARA's effort to build and support clinical research infrastructure, such as the CCRN in FA, has allowed us to leverage knowledge, outcomes and investigator experience from the FA natural history study and ACTIMMUNE Phase 2 study to enable us to advance Phase 3 study plans with shorter timelines and confidence. FARA's advocacy efforts have paved the way for us and others in the field, as FARA is well-known and respected in the rare disease community and specifically with key government agencies, such as the FDA. These efforts have been invaluable to optimizing our interactions and outcomes.” *Jeffrey W. Sherman, MD, FACP; Chief Medical Officer & Executive Vice President, Research & Development, Horizon Pharma plc.*

FARA is proud to have worked with the following companies:



FARA's non-profit partners include:





Infrastructure for FA Research

Disease Basics: Friedreich's ataxia (FA) is a debilitating, life-shortening, degenerative neuromuscular disorder affecting about fifteen thousand people globally. Onset of symptoms can vary from childhood to adulthood. Childhood onset of FA is usually between the ages of 5 and 15 and tends to be associated with a more rapid progression. Patients with FA typically develop a loss of coordination (ataxia) in their arms and legs, life shortening heart conditions, fatigue, scoliosis, slurred speech, diabetes, vision impairment and hearing loss. Not all patients will develop all symptoms, and they typically develop over time.

There are currently no approved treatments nor cures for FA. However, FARA has established the supporting infrastructure necessary to advance potential therapeutics. This includes cell and animal models, tissue collections, clinical infrastructure and registry data as detailed below.

Clinical Network: The Collaborative Clinical Research Network in Friedreich's Ataxia (CCRN in FA) is a growing international network, currently of a dozen clinical research centers that work together to advance treatments and clinical care for individuals with FA. Locations include Children's Hospital of Philadelphia, University of California Los Angeles, University of Chicago and University of South Florida in the USA along with sites in Australia and Canada, with an additional site in Brazil being added soon.

The network has been in place over 10 years, and has been involved in much of the critical clinical research in FA, including natural history studies, end point development, and clinical trials. The network has an outstanding reputation as a partner with all outside entities, as Reata notes below:

“The investigators and coordinators in the FA Collaborative Clinical Research Network are incredibly responsive and professional. With their partnership and FARA leadership, we were able to identify interested investigators and sites, develop a phase 2/3 protocol, obtain regulatory and ethics committee approvals, and launch a study in approximately six months. Once the study opened for screening, the first cohort of patients was identified, screened and enrolled in less than two months.”

Colin J. Meyer, M.D.
Vice President, Product
Development
Chief Medical Officer
Reata Pharmaceuticals, Inc.

Natural History: The CCRN in FA has collected natural history data on over 700 FA patients. The natural history for roughly 100 patients goes back more than 10 years. This data is available

on request for data mining to answer questions on clinical protocol design, end points, biomarkers etc. As patients seen in the network have been followed for some time, it may be possible to use those patients' natural history data to expand the interpretation of data collected in clinical trials.

Registry: FARA created and maintains the largest worldwide registry of FA patients. The registry currently has more than 2,000 registered patients. FARA has successfully recruited patients for 7 trials, representing all three phases of clinical trials, and can do so quickly – we filled an adult phase 2 trial of 60 patients in only 3 hours recently. Most FA trials recruit within only a few weeks rather than the months or years sometimes seen in other diseases.

Common Data Elements: The National Institute of Neurological Disorders and Stroke (NINDS), National Institutes of Health (NIH), has developed FA Common Data Elements (CDEs) for use in clinical research. These are now ready for use by the Friedreich's Ataxia clinical research community, and include measures of ataxia and performance, cardiac and clinical outcomes, potential biomarker information, demographic data, medical history etc. FARA encourages all clinical studies to incorporate these elements.

Clinical Endpoints and Biomarkers: The CCRN in FA has developed and validated the Friedreich's Ataxia Rating Scales (FARS) and several performance measures which are recognized disease relevant endpoints by FDA and are being used in FA clinical trials. Measurements of frataxin in blood and buccal swabs have been developed as a primary biomarker, and a new initiative to delve more deeply into potential biomarkers for different purposes is now underway..

Animal Models: FARA has supported the Jackson Laboratory to bring in existing mouse models of the disease, to fully characterize those models, and to develop additional models to meet the needs of the community. Nine mouse models are now available for use, and FARA and Jackson Labs are working together to develop new models that should be available soon. Current models include mice with mutations in the frataxin gene, which produce relatively low levels of mouse frataxin, as well as “knock in / knock out” mice, where the mouse gene has been knocked out and the human gene knocked in. Different mice express different levels of human and mouse frataxin, and therefore are appropriate for different purposes. Jackson Labs has characterized the mice in detail, and they or FARA can help determine which mouse is best for specific purposes, and what phenotypes are seen in which models (see details on available mice in the attached sheet). Also there is a webinar that summarizes the models and JAX characterization of those models that can be accessed at the following link:

<https://www.youtube.com/watch?v=asOkP6of7dE>

Cell Lines: FARA is working closely with investigators who are developing FA neuronal and cardiac cell models by differentiation of induced pluripotent stem (iPS) cells derived from patient fibroblasts. FARA has supported the collection of various cell lines from patients and carriers that are stored at the Coriell Cell Repository. This collection includes 56 lines, mostly B-lymphocyte lines (52) from affected and unaffected members of FA families. These come from people from 10 to 92 years of age, with repeat lengths from normal alleles through to 1282. Two fibroblast and two iPS lines are also available.

<http://ccr.coriell.org/Sections/Search/Search.aspx?PgId=165&q=frda>

There are more than 35 fibroblast lines available through Dr. Dave Lynch, Children's Hospital of Philadelphia, and Dr. Marek Napierala at the University of Alabama. All lines have been

newly created and genotyped. There are all lengths of GAA repeat including lines with >800 repeats and a few lines with point mutations.

Biological Samples: Through the CCRN in FA, small biorepositories of DNA, RNA, plasma and serum from FA patients have been established. Investigators in the CCRN in FA are willing to collaborate with researchers who need fresh biological samples from FA patients for translational and clinical research studies. In addition, FARA supports an autopsy and tissue donation program at the VA Medical Center in Albany, New York. This tissue bank has fixed and frozen tissues from brain, spinal cord, heart, sural nerve, and pancreas of 30 individuals with FA.

High Throughput Assays: Researchers have developed high throughput assays for drug discovery in FA. These assays vary significantly – for example some assays focus on readouts of mitochondrial function, while others focus on direct measurements of frataxin (e.g. genetically-derived assays that carry the expanded GAA repeats in the FRDA gene). FARA can put you in touch with researchers with the best assays for your purposes.

Mouse Models in Friedreich's Ataxia

FARA has supported the Jackson Laboratory (JAX) to bring in existing mouse models of the disease, to fully characterize those models, and to develop additional models to meet the needs of the community. JAX has ongoing work to develop and make available to the community new KO and humanized transgenics which could be available in 2015. Other mouse models are available through INSERM in France and UCLA in Los Angeles, CA. Nine mouse models are now available for use at Jackson Laboratories, and two additional models are available from INSERM:

B6.Cg-*Fxn*^{tm1Mkn} Tg(FXN)YG8Pook/J

Mice that are homozygous for the *Fxn*^{tm1Mkn} (frataxin) targeted allele and hemizygous for the Tg(FXN)YG8Pook (frataxin, human) transgene, display an age dependent, tissue specific expansion of the GAA repeat, with expansion accumulation in the CNS (particularly cerebellum), similar to the human pathology of Friedreich Ataxia. This strain is maintained heterozygous for the targeted mutation and hemizygous for the transgene.

<http://jaxmice.jax.org/strain/012253.html>

B6.Cg-*Fxn*^{tm1Mkn} *Fxn*^{Tm1Pand}/J

Animals bearing a (GAA)₂₃₀ expansion repeat “knock in” targeted to the endogenous *Fxn* locus coupled with an *Fxn* *Fxn* targeted “knock out” mutation allele disrupting exon 4, known as knockin/knockout mice or “KIKO” mice are viable and fertile. Analysis of frataxin levels in tissues from KIKO mice demonstrate a reduction of frataxin to 25-36% of wildtype controls. KIKO animals up to 1 year of age perform equivalent to wild type controls on rotarod test. Total iron concentration in tissues was similar in KIKO mice and their wild type littermates except in pancreas, where iron levels were significantly lower in KIKO mice. No iron deposits and only mild collagen staining around the vessels of the heart were observed in both year old KIKO mice and wild type controls. In contrast to FRDA patients, no detectable change in GAA repeat size was found over six studied generations, moreover, no evidence of somatic cell instability was noted as GAA repeat expansion size was the same in all analyzed tissues.

<http://jaxmice.jax.org/strain/014162.html>

B6.129-*Fxn*^{tm1.IPand}/J

Similar to stock [008470](#), these mice express a (GAA)₂₃₀ expansion repeat from the endogenous *Fxn* locus. Homozygotes produce an average of 75% of wild-type levels of frataxin protein, as assayed by Western blot densitometry analysis. The GAA repeat size was

found to be stable over the 6 generations studied. Mice that are homozygous for the targeted mutation are viable, fertile, normal in size and do not display any gross physical or behavioral abnormalities. These mice do not carry the neo selection cassette. This mutant mouse strain may be useful in studies of Friedreich's ataxia.

<http://jaxmice.jax.org/strain/011113.html>

B6.129-*Fxn*^{tm1Pand}/J

These mice express a (GAA)₂₃₀ expansion repeat from the endogenous *Fxn* locus. Homozygotes produce an average of 75% of wild-type levels of frataxin protein, as assayed by Western blot densitometry analysis. The GAA repeat size was found to be stable over the 6 generations studied. Mice that are homozygous for the targeted mutation are viable, fertile, normal in size and do not display any gross physical or behavioral abnormalities. This mutant mouse strain may be useful in studies of Friedreich's ataxia.

<http://jaxmice.jax.org/strain/008470.html>

***Fxn*^{tm1Mkn} Tg(FXN)YG8Pook/J**

Mice that are homozygous for the *Fxn*^{tm1Mkn} (frataxin) targeted allele and hemizygous for the Tg(FXN)YG8Pook (frataxin, human) transgene, display an age dependent, tissue specific expansion of the GAA repeat, with expansion accumulation in the CNS (particularly cerebellum), similar to the human pathology of Friedreich Ataxia. This strain is maintained heterozygous for the targeted mutation and hemizygous for the transgene.

<http://jaxmice.jax.org/strain/008398.html>

***Fxn*^{tm1Mkn} Tg(FXN)YG22Pook/J**

Mice that are homozygous for the *Fxn*^{tm1Mkn} (frataxin) targeted allele and hemizygous for the Tg(FXN)YG22Pook (frataxin, human) transgene, display an age dependent, tissue specific expansion of the GAA repeat, with expansion accumulation in the CNS (particularly cerebellum), similar to the human pathology of Friedreich Ataxia. Mice that are homozygous for the targeted allele and hemizygous for the transgene exhibit progressive retinal degeneration, impaired and decreased locomotor activity and coordination, an increase in body weight, and neurodegeneration. This strain is maintained heterozygous for the targeted mutation and hemizygous for the transgene. <http://jaxmice.jax.org/strain/010963.html>

B6.Cg-*Fxntm1Mkn* Tg(FXN)YG22Pook/J

The YG22 transgenic founder line carries a single copy of the human *FXN* gene with one GAA trinucleotide repeat sequence of 190 repeats. High levels of human *FXN* gene product (mRNA and protein) are detected, and 40-50% of the endogenous mouse *Fxn* gene product (protein) in mice heterozygous for the targeted mutation alone. Mice that are homozygous for the targeted allele and hemizygous for the transgene exhibit an age dependent, tissue specific expansion of the GAA repeat, with expansion accumulation in the CNS (particularly cerebellum), similar to the human pathology. The GAA triplet repeat exhibits intergenerational instability. Mice that are homozygous for the targeted allele and hemizygous for the transgene exhibit progressive retinal degeneration, impaired and decreased locomotor activity and coordination, and an increase in body weight. At 9 months

of age, muscle strength is decreased. Although, histological analysis reveals progressive vacuolation of the dorsal root ganglia, no abnormalities or loss of cerebellar Purkinje cells were detected. Iron deposition in cardiac tissue of 14-18 month old mice is observed. Dorsal root ganglia neurons contain lipofuscin deposits, and large axons are swollen and exhibit secondary demyelination. Cardiomyocytes contain lipofuscin deposits, free glycogen accumulation and disrupted mitochondria. Mitochondrial respiratory chain function is decreased and oxidized protein levels are increased.

<http://jaxmice.jax.org/strain/012910.html>

B6.Cg-Fxntm1.1Pand Fxntm1Mkn/J

These frataxin knock-in/knockout animals (KIKO Δ neo) mice harbor one allele of the frataxin (GAA)₂₃₀ Δ neo expansion mutation (*Fxntm1.1Pand*) on one chromosome, and one allele of the frataxin exon 4-deleted mutation (*Fxntm1Mkn*) on the homologous chromosome. This is similar to frataxin KIKO mice (Stock No. 014162), with the exception that these KIKO Δ neo mice have the (GAA)₂₃₀ expansion allele with the neo selection cassette removed. Characterization of KIKO mice performed at The Jackson Laboratory revealed that starting at 6 months of age, these animals exhibit an abnormal "weaving" gait when subjected to a forced treadmill walk. This phenotype occurs with increasing penetrance as the mice age.

<http://jaxmice.jax.org/strain/012329.html>

B6.Cg-Tg(FXN)1Sars Fxntm1Mkn/J

Mice that are hemizygous for the transgene and homozygous for the targeted mutation are viable, normal in size and do not display any gross physical or behavioral abnormalities. The Donating Investigator reports that no other behavioral or histological phenotype has been observed. FISH and karyotyping shows one copy of the transgene inserted on Chromosome 5.

<http://jaxmice.jax.org/strain/008586.html>

Additional mouse models have been created by Helene Puccio's laboratory at INSERM in France; these may be accessed through Dr. Puccio, contact through FARA:

Puccio FXN conditional knockouts - cardiac and neuronal knockouts

The cre-loxP recombination system was used to perform deletion of Fxn exon 4 from a conditional floxed allele (FxnL3) using tissue-specific, or tissue-specific and inducible, Cre mouse lines. Cardiac-specific (MCK-Cre) and neuronal (NSE-Cre, Prp-CreERT) models of FRDA were obtained (Puccio et al., 2001; Simon et al., 2004). Together, these models reproduce most of the characteristic features of the disease, including hypertrophic cardiomyopathy, progressive spinocerebellar and sensory ataxia. Time-dependent molecular and functional dissection of the MCK-Cre mouse model showed that frataxin deficiency primarily affects Fe-S cluster-containing enzymes (Puccio et al., 2001). The Fe-S cluster deficit is observed before the first evidence of cardiac dysfunction, and before the characteristic mitochondrial iron accumulation. Echocardiography demonstrated that MCK-Cre mice develop a progressive left ventricular hypertrophy that rapidly associates with

geometric remodeling (dilatation) leading to cardiac failure, consistent with the natural history of the human disease (Seznec et al., 2004; Regner et al., 2011). Interestingly, no evidence of oxidative damage was observed in this model, suggesting that the formation of reactive oxygen species (ROS) is not essential in the early onset of the disease (Seznec et al., 2005). Review: <http://www.ncbi.nlm.nih.gov/pubmed/17203663>,
Neuronal mouse: <http://www.ncbi.nlm.nih.gov/pubmed/14985441>
Cardiac mouse: <http://www.ncbi.nlm.nih.gov/pubmed/11175786>

An additional mouse model has been created by Vijay Chandran in Daniel Geschwind's laboratory at UCLA; anticipated availability early 2015 through UCLA, contact through FARA:

UCLA mice - Inducible frataxin knockdown mice

The UCLA mice have a dox-inducible RNAi that knocks down frataxin in a tissue specific manner. Characterization to date suggests that the mice have many FA-like phenotypes, and characterization is continuing. These mice should be available through UCLA in early 2015.

New Models: In addition, with Jackson Laboratories, we are embarking on new mouse models that may be available in late 2015.

- New Frataxin exon 1 knockout mouse.
- New transgenic with human gene from a patient with severe phenotype and non-interrupted repeats inserted into an exon 1 knockout
- New conditional knockout mice.
- New mice with increased, uninterrupted repeat sequence insertions into the human gene.