Mouse Models in Friedreich's Ataxia

FARA is grateful to the FA researchers who have created and characterized FA mouse models. FARA collaborates with the Jackson Laboratory (JAX), Brunel University (UK), Erasme University (Belgium), Murdoch Children's Research Institute (Australia), INSERM (France), and UCLA (USA) to make mouse models of Friedreich's ataxia (FRDA) available to the greater research community. FARA has partnered with JAX to centralize and expedite sharing of existing FA mouse models and to characterize those models. In addition, JAX has ongoing work to develop and make new models available to the Friedreich's ataxia community. Most mouse models are now available for use at Jackson Laboratories, and three knock-out models are also available from Helene Puccio and one through UCLA (Chandran et al Inducible and reversible phenotypes in a novel mouse model of Friedreich's Ataxia. Elife 2017). In addition, FARA is aware of several additional models that are being created and tested, including those described in Fil et al (Mitochondrial damage and senescence phenotype of cells derived from a novel frataxin G127V point mutation mouse model of Friedreich's ataxia. Dis Model Mech. 2020) and Salami et al 2020 (Stress-induced mouse model of the cardiac manifestations of Friedreich's ataxia corrected by AAV-mediated gene therapy. Human Gene Therapy, 2020).

These mouse models have been created by several different techniques and vary in degree of protein reduction, robustness of a phenotype, tissues involved and age of onset of any phenotype. Below is a table listing potential uses and suggestions on the *in vivo* models that might be best suited for each specific application, followed by a detailed description of each FA model.

Potential uses	Models	Name	Supplier
Therapeutic approaches that upregulate FXN mRNA or protein, frataxin bypass interventions and other therapeutic approaches downstream of frataxin function	Mice with both Fxn alleles knocked out and transgenic for the human *FXN* gene with an expanded GAA repeat sequence Mice with a repeat sequence inserted into the mouse gene	YG8R, YG22R, YG8sR YG8 800 KIKO, KIKI	JAX
Gene therapy or frataxin replacement therapy and studies on frataxin loss in specific tissues	Conditional knock-out mouse models	MCK-Cre, NSE-Cre, PVALB-Cre $Fxn^{flox/null}$:MCK-Cre, $Fxn^{flox/null}$::PV-Cre	Helene Puccio (exon 4 deleted, MTA with INSERM) JAX (exon 2 deletion)

Temporal studies on FXN knock-down phenotype	Inducible knock-down	FRDAkd	UCLA
I*n vivo* impact of the GAA repeats on silencing mechanisms	Mice with the mouse allele knocked out and transgenic for the human *FXN* gene with an expanded GAA repeat sequence Mice with a repeat sequence inserted into the mouse Fxn gene	YG8R, YG22R, YG8sR YG8 800 KIKO, KIKI	JAX
Studies on repeat instability	Mice with the mouse allele knocked out and transgenic for the human *FXN* gene with an expanded GAA repeat sequence	YG8R, YG22R, YG8sR YG8 800	JAX

Mice with the human gene inserted (i.e. human gene with a GAA repeat sequence):

These mice are suitable for looking at frataxin upregulating compounds, and may be useful for compounds that act downstream of FXN and, possibly, for frataxin replacement strategies. Mark Pook (Brunel University, UK) created a humanized model containing a YAC carrying a human FXN gene with a repeat sequence ("Pook mice"), which is available on a background of a mouse where the mouse fxn gene has been deleted. The YG8-derived strains show repeat instability, but limited detectable phenotype. The three YAC transgenic mouse models (YG8R, YG22R and YG8sR) show a progressive decrease in the motor coordination. All three models exhibit GAA repeat somatic instability in the brain, cerebellum and liver, as well as exhibited glucose intolerance and insulin hypersensitivity. The greatest FXN deficiency of the three models tested was in YG8sR.

Pook YG8R model. This human FXN YAC transgenic mouse model has the mouse frataxin knockout allele (*Fxn*$^{-}$) and the human FXN YAC transgene from founder YG8, and carries two copies of the human FXN gene with ~82 and ~190 GAA repeats. Mice homozygous for the Fxn- knockout allele and hemizygous for the YG8 transgene, called YG8R mice, are rescued from knockout lethality and have transgene expression that results in an age-dependent, tissue-specific expansion of the GAA repeat, with expansion accumulation observed in the CNS (particularly cerebellum), similar to the human pathology of Friedreich's Ataxia. The GAA triplet repeats exhibit intergenerational instability. As this model recapitulates the epigenetic landscape, it is particularly useful to test molecules that act on the GAA repeat or the epigenetic modifications. This model has no overt ataxia phenotype and has overall limited phenotype before 6-12 months of age.
Note: At JAX this strain is maintained heterozygous for the Fxn deletion and hemizygous for the transgene. Mice singly homozygous for the FXN global null allele are embryonic lethal.
These mice are available on two genetic backgrounds:
Fxn^{tm1Mkn}Tg(FXN)YG8Pook/J (https://www.jax.org/strain/008398) original Pook mouse with mixed genetic background

B6.Cg-*Fxn*^{tm1Mkn} Tg(FXN)YG8Pook/J (https://www.jax.org/strain/012253) backcrossed to C57BL/6J for five generations
Description at:
https://www.ncbi.nlm.nih.gov/pmc/?term=PMC2842930
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4157886/

Pook YG22R model. This human FXN YAC transgenic mouse model has the mouse frataxin knockout allele (*Fxn*$^{-}$) and the FXN YAC transgene from founder YG22 (carrying a single copy of the human FXN gene with ~190 GAA trinucleotide sequence repeats). Mice homozygous for the knockout and hemizygous for the YG22 transgene, called YG22R mice, are rescued from knockout lethality and have transgene expression that models the phenotype of Friedreich's Ataxia (FRDA). Various phenotypes have been reported for these mice by different groups. These mice 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. These strains are maintained heterozygous for the targeted mutation and hemizygous for the transgene and are available on two genetic backgrounds:
B6.Cg-Fxn^{tm1Mkn}Tg(FXN)YG22Pook/J (http://jaxmice.jax.org/strain/012910)
Fxn^{tm1Mkn}Tg(FXN)YG22Pook/J (https://www.jax.org/strain/010963)
Description at: https://www.ncbi.nlm.nih.gov/pubmed/?term=PMC2842930
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4157886/

Pook YG8sR model. This human FXN YAC transgenic mouse model has the mouse frataxin knockout allele (*Fxn*$^{--}$) and the human FXN YAC transgene single repeat YG8s, in which one of the two copies of the human FXN gene was lost. GAA repeat size in this mouse is ~250-300. Mice homozygous for the knockout are rescued from lethality by the expression of the YG8s transgene are called YG8sR mice. Compared to YG8R, the YG8sR model is characterized by lower level of frataxin. The YG8s transgene exhibits somatic GAA repeat instability.
Description at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4348561/
Fxn^{tm1Mkn}Tg(FXN)YG8Pook/2J (https://www.jax.org/strain/024113)

Pook YG8 800 Fxn^{null}::YG8s(GAA)$_{>800}$. Fxn^{null}::YG8s(GAA)$_{>800}$ mice are a human FXN YAC transgenic mouse model harboring a global null allele of mouse frataxin (Fxn^{null}) and the human FXN YAC transgene single repeat YG8s with a GAA repeat size of >800 . As of December 2017, the GAA repeat values were in the 800-899 range - please inquire with JAX about the current GAA repeat size. As of November 2018, JAX breeding of hemizygous mice with noncarrier mice results in 58% of offspring that are hemizygous, and 80% of those hemizygotes stay in the 775-900 range. The phenotype characterization of Fxn^{null}::YG8s(GAA)$_{>800}$ has not been completed to date. However, they may be expected to have a phenotype similar to that of YG8sR mice (Stock No. 024113). Of note, The Jackson Laboratory Stock No. 030395 colony reports the majority of Fxn^{null}::YG8s(GAA)$_{>800}$ mice exhibit hair loss, even when singly housed. A control strain is available with the same Fxn^{null} allele and a single copy integration of the Y47 human FXN YAC transgene encoding human frataxin with normal-sized (GAA)$_9$ repeats: Fxn^{null}::Y47. Note that the Fxn KO allele in this strain was created in house by JAX (exon 2 deletion Fxnem2.1Lutzy) and is different from the YG8R and YG8sR (exon 4 deletion)
Description at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4348561/
Fxn$^{em2.1Lutzy}$ Tg(FXN)YG8Pook/800J (https://www.jax.org/strain/030395)

Sarsero model. Joseph Sarsero (Murdoch Children's Research Institute, Australia) created a humanized model by inserting the FXN human sequence with 500 interrupted GAA repeats on a bacterial artificial chromosome (BAC) in the mouse genome. These Tg(FXN);Fxn- mice harbor the the FXN*500GAA transgene (Tg(FXN)1Sars) and frataxin knockout allele (*Fxn*^{tm1Mkn}). The FXN*500GAA transgene was found to have an interrupted 500 GAA trinucleotide repeat inserted into intron 1. This transgene was injected into C57BL/6J donor eggs. FISH and karyotyping of mice from founder line 1 shows one copy of the transgene inserted on Chromosome 5. These mice have much lower frataxin levels than control mice (~10% expression of wild type frataxin), but no overt behavioral phenotype has been identified. These mice might not be suited for gene reactivation studies because the GAA repeat is interrupted, unlike the human condition, and the mechanism of silencing is unclear.
Description at https://www.cell.com/molecular-therapy-family/molecular-therapy/pdf/S1525-0016(05)01223-2.pdf
B6.Cg-Tg(FXN)1Sars *Fxntm1Mkn*/J (http://jaxmice.jax.org/strain/008586).

Mice with a repeat sequence inserted into the mouse gene:

Massimo Pandolfo (Erasme University, Belgium) was able to insert GAA repeats into the endogenous mouse Fxn gene. Mice heterozygous for the GAA insertion and harboring a Fxn knockout allele (KIKO) are suitable for looking at compounds that act downstream of FXN, or compounds that replace FXN. Subtle behavioral phenotypes have been observed in the KIKO mice at about 1 year of age, and biochemical and physiological changes can be detected much earlier.

Pandolfo KIKO model. These frataxin knock-in/knockout (KIKO) mice harbor one allele of the frataxin $(GAA)_{230}$ expansion mutation (*Fxn*^{tm1Pand}) on one chromosome, and one allele of the frataxin exon 4-deleted mutation (*Fxn*^{tm1Mk}) on the homologous chromosome. 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 wildtype controls on rotarod test. Total iron concentrations were similar in all tested tissues of KIKO and wildtype mice except in pancreas: KIKO mice demonstrate lower iron levels in pancreatic tissues. No iron deposits and only mild collagen staining around the vessels of the heart were observed in both year old KIKO mice and wildtype controls. In contrast to Friedreich's Ataxia 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. However, 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.
JAX distributes two versions of this model, with and without the neomycin selection cassette
B6.Cg-*Fxn*^{tm1Mkn}*Fxn*^{Tm1Pand}/J (https://www.jax.org/strain/014162)
B6.Cg-*Fxn*^{tm1Mk}*Fxn*^{tm1Pand}/J (https://www.jax.org/strain/012329)
Description at: https://pubmed.ncbi.nlm.nih.gov/11852098/
Phenotype described in: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5719255, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5051948/, https://pubmed.ncbi.nlm.nih.gov/29259026/, https://pubmed.ncbi.nlm.nih.gov/32269244/, https://pubmed.ncbi.nlm.nih.gov/31974344/

Pandolfo frda$^{230GAA/230GAA}$ or "KIKI" (knockin/knockin) model. 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.
JAX distributes two versions of this model, with and without the neomycin selection cassette
B6.129-*Fxn^{tm1Pand}*/J (http://jaxmice.jax.org/strain/008470)
B6.129-*Fxn$^{tm1.1Pand}$*/J (http://jaxmice.jax.org/strain/011113)
Description at: https://febs.onlinelibrary.wiley.com/doi/abs/10.1016/S0014-5793%2802%2902251-2

Frataxin knock-out mouse models:

Hélène Puccio (IGBMC/INSERM and Université de Lyon, France) has created and characterized multiple conditional knock-out mouse models. These animals are perhaps best for gene and protein replacement strategies and to understand tissue-specific downstream events of frataxin deficiency. This is because the endogenous gene is ablated to recapitulate an FRDA-like phenotype, although to a more severe extent, since there is a complete absence of frataxin in the tissues of interest. The team had previously shown that full frataxin knockout is embryonic lethal at E6.5 days, demonstrating that frataxin is an essential protein.

Puccio FXN conditional knockout models.
These are conditional knockout mice, where frataxin may be knocked out in specific tissues when the Cre recombinase is expressed. Mice have severe cardiac or neuronal phenotypes. The cre-loxP recombination system was used to make a conditional allele of the mouse *Fxn* exon 4 from (Fxn^{L3}). The exon 4 deleted allele is denoted Fxn^{L-}. To obtain the conditional knockout (cKO), mice heterozygous for the deleted allele $Fxn^{+/L-}$ and carrying a tissue-specific Cre transgene is crossed with a mouse homozygous for the conditional allele ($Fxn^{L3/L3}$). The conditional mutant animals bear the following genotype: $Fxn^{L3/L-}$; $TgCre^{+}$. Cardiac-specific (MCK-Cre) and neuronal (NSE-Cre, Prp-CreERT) models of FRDA are available (Puccio et al., 2001; Simon et al., 2004). More, recently a Parvalbumin cKO which has more of the CNS specific phenotype associated with FA was also generated (Piguet et al. 2018). Beta-cell and liver specific conditional knockout have also been generated.
Review: http://www.ncbi.nlm.nih.gov/pubmed/17203663;
Neuronal mouse model: http://www.ncbi.nlm.nih.gov/pubmed/14985441;
https://www.ncbi.nlm.nih.gov/pubmed/29853274
Cardiac mouse model: http://www.ncbi.nlm.nih.gov/pubmed/11175786

Parental lines to obtain Frda $^{L3/\Delta;}$ MCK-Cre+ (MCK-CRE) and Frda $^{L3/\Delta}$; NSE39-Cre+ (NSE-CRE) are available from Helene Puccio. An MTA needs to be signed with INSERM.

Jackson Labs has created an in-house exon 2 FXN knockout model ($Fxn^{em2.1Lutzy}$) different from Dr. Koenig and Dr. Puccio's FXN exon 4 knockout to create the following conditional KO models:

Fxn$^{flox/null}$:MCK-Cre. These mice harbor a Cre- conditional frataxin allele, a frataxin global knockout allele and a cardiac/skeletal muscle-specific Cre recombinase transgene. These mice show a strong cardiac

phenotype. Due to early-onset cardiomyopathy, $Fxn^{flox/null}$::MCK-Cre are distributed at 4-5 weeks old. JAX distributes phenotypically-normal parental lines:

C57BL/6J-Fxnem2Lutzy/J (https://www.jax.org/strain/028520)
Fxn^{flox} homozygous frataxin floxed exon 2
B6.Cg-$Fxn^{em2.1Lutzy}$ Tg(Ckmm-cre)5Khn/J (https://www.jax.org/strain/029100)
Fxn^{null}::MCK-Cre. These mice harbor a frataxin global knock-out allele and a cardiac/skeletal muscle specific Cre recombinase transgene. These double mutant mice are "phenotypically normal"
B6.Cg-$Fxn^{em2Lutzy}Fxn^{em2.1Lutzy}$Tg(Ckmm-cre)5Khn/J (https://www.jax.org/strain/029720)

$Fxn^{flox/null}$::PV-Cre These mice have a Cre-conditional frataxin allele, a global knockout frataxin allele and a parvalbumin neuron-specific Cre recombinase knockin allele. Due to early-onset ataxia, Fxnflox/null::PV-Cre are distributed at 4-7 weeks of age. JAX distributes the phenotypically-normal parental lines:

C57BL/6J-Fxnem2Lutzy/J (https://www.jax.org/strain/028520)
Fxn^{flox} homozygous frataxin floxed exon 2
B6.Cg-$Pvalb^{tm1(cre)Arbr}$ $Fxn^{em2.1Lutzy}$/J (https://www.jax.org/strain/030218)
Fxn^{null}::PV-Cre These mice harbor a frataxin global knockout allele and a Pvalb knock-in allele directing Cre recombinase expression to parvalbumin-expressing neurons. These mice are phenotypically normal and are a parental control used to generate the progressive ataxia mouse line $Fxn^{flox/null}$::PV-Cre.
B6.Cg-$Pvalb^{tm1(cre)Arbr}$ $Fxn^{em2Lutzy}$ $Fxn^{em2.1Lutzy}$/J (https://www.jax.org/strain/029721)

Frataxin inducible knock-down mouse model:

These animals were created by Vijay Chandran in Daniel Geschwind's laboratory at UCLA and could be used for therapeutic strategies that do not involve increasing endogenous frataxin. This is because an inducible shRNA knocks down *Fxn* mRNA to recapitulate an FRDA-like phenotype. The endogenous gene in this model does not have GAA repeats. They are available through UCLA, https://techtransfer.universityofcalifornia.edu/NCD/24493.html.

UCLA mouse model. Chandran created an FRDA mouse model, by genomic integration of a single copy of an Fxn-targeting shRNA transgene (doxycycline-inducible) under the control of H1 promoter in the rosa26 genomic locus. These mice can be induced to conditionally knock down frataxin to low levels using doxycycline to induce the expression of the Fxn-targeted shRNA. This mouse model displays a phenotype similar to FRDA patients, including cardiac hypertrophy, elevated iron-responsive proteins, neurodegeneration, motor neuropathy, scoliosis, and ataxia. On removal of dox (and increased levels of frataxin) mice can recover most functions. The physiology and molecular characterization of this recovery is still under investigation and not fully understood.
Description of the model: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5736353/

For inquiries about the JAX mouse models, please contact Cathleen Lutz, PhD (cat.lutz@jax.org).