

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), IGBMC (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. Twelve mouse models are now available for use at Jackson Laboratories, and three additional models are available from IGBMC, and one through UCLA.

A 2013 review of mouse models for FRDA is at <https://www.ncbi.nlm.nih.gov/pubmed/23859342>

Summary of mouse models – details below.

Type of Model	Name	Description
Mice deleted for the endogenous frataxin gene carrying the human locus (i.e. human gene with a repeat sequence):	YG8	$Fxn^{tm1Mkn}Tg(FXN)YG8Pook/J$
	YG8R	$Fxn^{tm1Mkn}Tg(FXN)YG8Pook/J$
	YG22	$Fxn^{tm1Mkn}Tg(FXN)YG22Pook/J$
	YG22R	$Fxn^{tm1Mkn}Tg(FXN)YG22Pook/J$
	YG8sR	$Fxn^{tm1Mkn}Tg(FXN)YG8Pook/2J$
	500GAA	$Tg(FXN)1Sars Fxn^{tm1Mkn}/J$
Mice with a repeat sequence inserted into the mouse gene:	KIKO	$Fxn^{tm1Mkn}Fxn^{tm1Pand}/J$
	KIKO Δ neo	$Fxn^{tm1Mk}Fxn^{tm1Pand}/J$
	KIKI	$Fxn^{tm1Pand}/J$
Frataxin knock-out mouse models:	MCK-Cre	Cardiac knockout
	MCK-Cre	$Fxn^{em2Lutzy}Fxn^{em2.1Lutzy}Tg(Ckmm-cre)5Khnl/J$
	NSE-CRE	Neuronal knockout
	Prp-CreERT	Neuronal knockout
	Parv-CRE	Neuronal knockout
Frataxin inducible knock-down mouse model:	shRNA FXN	Doxycycline inducible shRNA knockdown Fxn mRNA

Mice deleted for the endogenous frataxin gene carrying the human locus (i.e. human gene with a repeat sequence):

These mice are suitable for looking at frataxin upregulating compounds, and may be useful for compounds that act downstream of FXN and 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, which are available on a background of a mouse where the mouse frataxin has been deleted. The original YG8 and YG22 lines are available, where the repeat expands in some tissues. The YG8s strain has a stable repeat sequence. New lines with longer repeats are currently being generated and studied. Joseph Sarsero (Murdoch Children's Research Institute, Australia) created a humanized model by inserting the human sequence on a BAC on the knockout background; these mice have much lower frataxin levels, but no detectable phenotype.

- Fxn^{tm1Mkn} Tg(FXN)YG8Pook/J (<http://jaxmice.jax.org/strain/012253>)

YG8 model. This human FXN YAC transgenic mouse model has the mouse frataxin knockout allele (Fxn^{-}) and the human FXN YAC transgene from founder YG8 (carrying two tandem copies of the human FXN gene with ~82 and ~190 GAA trinucleotide sequence repeats). Mice that are heterozygous for the Fxn^{-} knockout allele and hemizygous for the YG8 transgene are viable and fertile. Human FXN gene product (mRNA or protein) has detected by RT-PCR and Western blot analysis. Approximately 40-50% of the endogenous mouse Fxn gene product (protein) is detected by Western blot analysis in mice heterozygous for the Fxn^{-} knockout allele alone. This mouse is useful to generate the YG8R mouse.

Description at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2842930/?report=reader>

- Fxn^{tm1Mkr} Tg(FXN)YG8Pook/J (<https://www.jax.org/strain/008398>)

YG8R model. 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 recapitulate 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.

Description at:

<https://www.ncbi.nlm.nih.gov/pmc/?term=PMC2842930>

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4157886/>

- Fxn^{tm1Mkr} Tg(FXN)YG22Pook/J (<http://jaxmice.jax.org/strain/010963>)

YG22 model. Mice that are heterozygous for the knockout allele (Fxn^{-}) and hemizygous for the Tg(FXN)YG22Pook (frataxin, human) transgene containing between 170–260 repeats, 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 knockout mutation and hemizygous for the transgene.

This is a parental strain

Description at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4157886/>

- Fxn^{tm1Mkr} Tg(FXN)YG22Pook/J (<http://jaxmice.jax.org/strain/012910>)

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.

Description at: <https://www.ncbi.nlm.nih.gov/pubmed/?term=PMC2842930>
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4157886/>

- *Fxn^{tm1Mkn}*Tg(FXN)YG8Pook/2J (<https://www.jax.org/strain/024113>)

YG8sR model. This human FXN YAC transgenic mouse model has the mouse frataxin knockout allele (*Fxn⁻*) and the human FXN YAC transgene small repeat YG8s (contracted integration to a single copy of the human FXN gene with GAA trinucleotide repeats [~250-300]). Mice homozygous for the knockout and hemizygous for the YG8s transgene, called YG8sR mice, are rescued from knockout lethality and have transgene expression that models the phenotype of Friedreich's ataxia (FRDA). Compared to other human FXN YAC transgenic rescue mouse models, the YG8sR model expresses a single copy of the human frataxin, resulting in much greater FXN deficiency. As such, the donating investigator concludes that YG8sR can be considered the most suitable YAC transgenic GAA repeat-based mouse model for the investigation of potential therapies for FRDA. This model is reported to show balance and grip strength phenotypes from 9-12 months of age.

Description at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4348561/>

- Tg(FXN)1Sars *Fxntm1Mkn*/J (<http://jaxmice.jax.org/strain/008586>)

500GAA model. 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 designed with a bacterial artificial chromosome (BAC) containing the entire human frataxin (*FXN*) gene with 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. ~10% expression of wild type frataxin. JAX has done extensive phenotyping but no overt behavioral phenotype has been identified.

Mice with a repeat sequence inserted into the mouse gene:

These mice are suitable for looking at compounds that act downstream of FXN, and possibly compounds that replace FXN. Massimo Pandolfo (Erasmus University, Belgium) was able to knock repeats into the endogenous mouse frataxin gene. Subtle behavioral phenotypes have been observed in these mice at about 1 year of age, and biochemical and physiological changes can be detected much earlier.

- $Fxn^{tm1Mkn}Fxn^{tm1Pand}/J$ (<http://jaxmice.jax.org/strain/014162>)

KIKO model. Animals bearing a (GAA)₂₃₀ expansion repeat “knock in” targeted to the endogenous *Fxn* locus coupled with an *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. As in Friedreich ataxia, epigenetics marks of silencing of the locus has been detected. 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. These mice may be useful for studying epigenetic targeting compounds.

Phenotype described in: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5719255>,

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5051948/>,

Description at: <https://febs.onlinelibrary.wiley.com/doi/abs/10.1016/S0014-5793%2802%2902251-2>

- $Fxn^{tm1Mk}Fxn^{tm1Pand}/J$ (<http://jaxmice.jax.org/strain/012329>)

KIKOΔneo model. These frataxin knock-in/knockout animals (KIKOΔneo) mice harbor one allele of the frataxin (GAA)₂₃₀Δneo 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 KIKOΔneo 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. Similar constructs under a different genetic background exist (<http://jaxmice.jax.org/strain/011113>).

Phenotype described at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5051948/>,

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5769605/> and

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5719255/>.

- $Fxn^{tm1Pand}/J$ (<http://jaxmice.jax.org/strain/008470>)

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.

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, 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 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. The conditional knockout allele is available through Dr. Puccio; please contact through FARA or directly.

- Cardiac and neuronal FXN knockouts

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. These mice are suitable for studying most treatment types. 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, mice heterozygous for the deleted allele *Fxn*^{+L-} and carrying a tissue-specific (or tissue-specific and inducible) Cre transgene is crossed with a mouse homozygous for the conditional allele (*Fxn*^{L3/L3}) mouse lines. 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 were obtained (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>

Jackson Labs has Dr. Koenig and Dr. Puccio's FXN exon 4 knockout available, as well as an exon 2 FXN knockout model created in-house (*Fxn*^{em2.1Lutzky}), and it's own cardiac conditional knockout as detailed below :

- *Fxn*^{m1Mkn}/J (<http://www.jax.org/strain/016842>)

Frda^{del4} model. Exon 4 deleted model generated by M. Koenig and H. Puccio. *Frda*^{del4} mice exhibit an embryonic lethal phenotype at E6.5.

- *Fxn*^{em2Lutzky}*Fxn*^{em2.1Lutzky}Tg(Ckmm-cre)5Khnl/J (<https://www.jax.org/strain/029720>)

***Fxn*^{flox/null}::MCK-Cre JAX model.** These mice conditionally knock out frataxin in cardiac and skeletal muscle where Cre is expressed. *Fxn*^{flox/null}::MCK-Cre mice harbor a Cre-conditional frataxin allele of exon 2, a frataxin exon 2 knockout allele and a cardiac/skeletal muscle-specific Cre recombinase transgene. These mice show a strong cardiac phenotype, but have not yet been published.

Frataxin inducible knock-down mouse model:

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

- **FRDAkd mouse model.** These mice can be induced to knock down frataxin to low levels using doxycycline. The vector contains an shRNA sequence against the mouse frataxin (*Fxn*) gene regulated by the H1 promoter with tet-operator sequences (tetO) and tet repressor (tetR) under the control of the CAGGS promoter. Transcription of the *Fxn* shRNA is blocked in cells expressing tetR. Upon induction by doxycycline (dox), tetR is removed from the tetO sequences inserted into the promoter, allowing transcription of shRNA against *Fxn*. shRNA expression leads to RNAi-mediated knockdown of the *Fxn* gene. This mouse model displays a phenotype parallel 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.

Description of the model: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5736353/>

Lastly, with JAX, we are embarking on new mouse models with increased, uninterrupted repeat sequence insertions into the human gene. For inquiries about the JAX mouse models, please contact Cathleen Lutz, PhD (cat.lutz@jax.org).



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