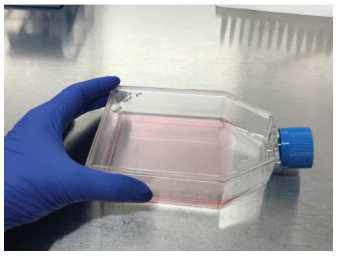
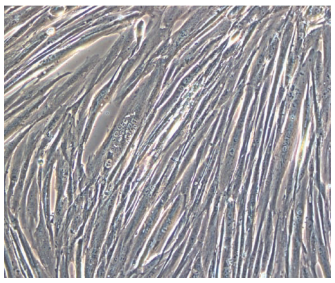
Porcine models for FSHD therapeutic development and preclinical testing (Pt 1)

Why do we need a large animal model for FSHD?

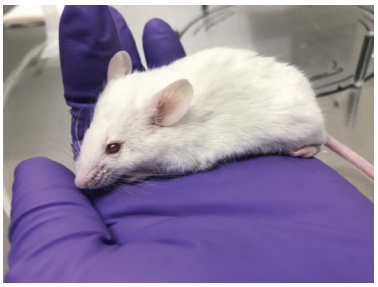




Lab grown human muscle cells

Single cell type grown in a 2D monolyer. Typically cultures of ~1,000-1,000,000 cells.

Drugs go to clinic without one, but is it a good idea? That depends on the drug.



Lab mouse <1oz (20-25g)

All types of cells.
3D cellular growth with organs, vascularization and innervation, (+/- immune system).
Highly inbred and genetically identical, which, while experimentally desirable, can be misleading.

Many drugs fail going from mouse to human.

Target "organism" ~50-300+lbs (~20-150+kg)

All types of cells.
3D cellular growth, with organs, vascularization, innervation, and an immune system.
Outbred (hopefully!) with >40 trillion cells.

Drug delivery, specificity, dosing, efficacy, and durability may vary greatly going from the lab mouse to the clinic.

Large animal models have more human-like musculature, organ systems, toxicology responses, immune systems and require more relevant drug dosing and delivery; however, there can be ethical concerns with some.

Dogs

Non-human primates (*e.g.*, African green monkeys)

Chimpanzees

Porcine models for FSHD therapeutic development and preclinical testing (Pt 2)

There is currently no large animal model for FSHD. Do we need a large animal model to get to clinic?

ICH (International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use) guidelines require the use of two species in non-clinical studies for pharmaceuticals: one rodent and one non-rodent species. With its many anatomical, physiological and functional similarities to humans, the minipig (*Sus scrofa*) is an excellent alternative to dogs or non-human primates for clinical safety testing, drug dosing and human disease modeling.

All experimental drugs or therapeutic interventions will need to be tested on an alternative to rodents.

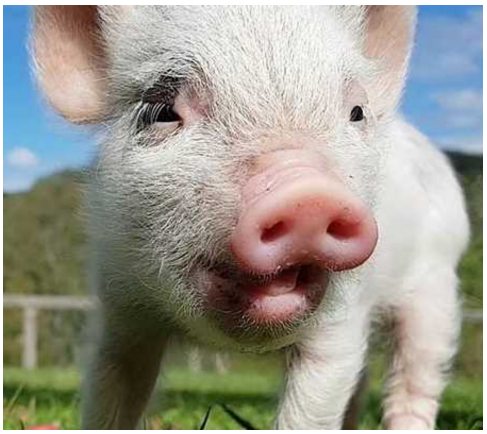
Straight toxicology and safety can be performed in a non-human primate, and that is the current approach in FSHD; however, these experiments give no information on drug efficacy.

Other neuromuscular diseases have canine models. For example, Duchenne muscular dystrophy (DMD) uses the GRMD (golden retriever muscular dystrophy) model, which has a spontaneous *DMD* mutation.

However, there are no natural or spontaneous animal models of FSHD; therefore, we must engineer animal models of FSHD. Since we are starting from scratch, we can choose the model system.

The Jones Lab at the University of Nevada, Reno is collaborating with industry to make large animal models of FSHD using Göttingen minipigs, which have many advantages over other large animal models.

Göttingen minipigs



- Small stature, ~77lbs or 35kg.
- Calm demeanor, not aggressive.
- Genetically and microbiologically defined.
- Patented genome with exclusive rights to each gene. The Jones lab has licensed the *DUX4* and *SMCHD1* genes.
- Piglets are generated by cloning (SCNT or somatic cell nuclear transfer), so there is no need to keep a breeding colony.
- Moderate gestation time (114 days) and early sexual maturity (boars 3-4 mo, sows 4-5 mo).
- Farm licensed for transgenic pigs and for performing the proper analysis and interventions (including AAV).
- Not a dog or primate!

Porcine models for FSHD therapeutic development and preclinical testing (Pt 3)

Pigs do not have the *DUX4* gene; therefore it must be inserted into their genome.

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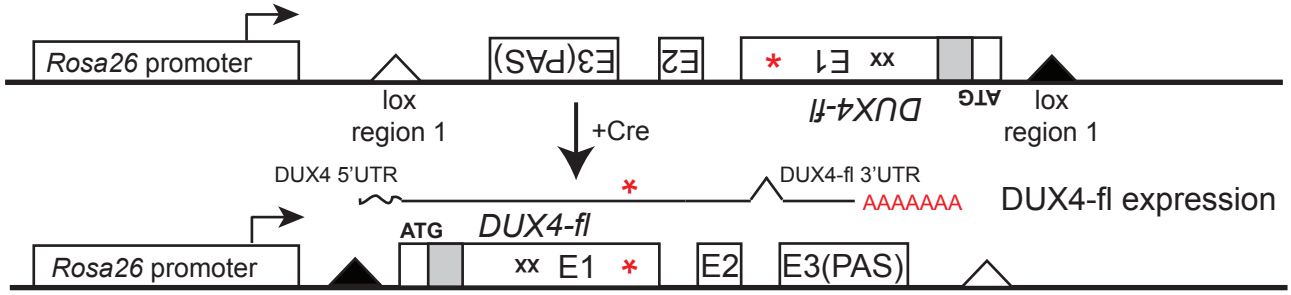
FSHD CANADA FOUNDATION
Curing the #1 Form of Muscular Dystrophy
FSHD Global Research Foundation Ltd
The Chris Carrino Foundation for FSHD
The William R. Lewis Family

Currently in production. Estimated availability in 2022.

A. DUX4 Minipig

Rosa26 (Ubiquitous expression locus)



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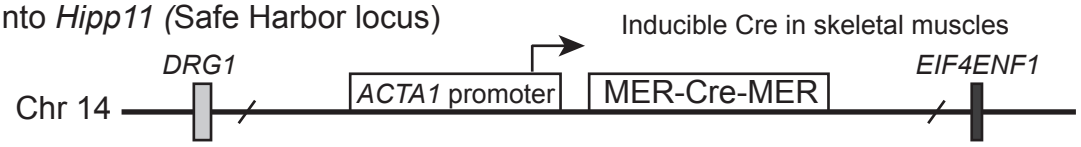
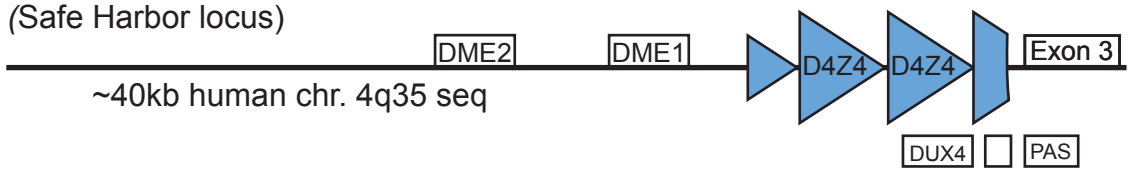
Into *Hipp11* (Safe Harbor locus)



B. FSHD1 Minipig

Into *Hipp11* (Safe Harbor locus)



C. FSHD1+2 Minipig FSHD1 pig + mut-*SMCHD1* (FSHD2)

Into *Hipp11* (Safe Harbor locus)

Planned (subject to technical capabilities) FSHD-like minipig models, male and female of each, will provide a large animal model for preclinical screening of virtually any FSHD therapeutic approach to assess dosing, efficacy, durability, and safety.