# Generation of Allelic Series using CRISPR/Cas9 to Study Familial ALS (and other Rare Diseases)

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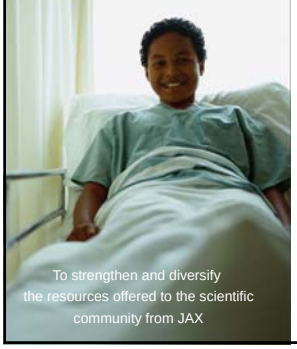
Athens November 2017

*Solutions for Fundamental Research and Therapeutic Development in Neurobiology and Rare Diseases.*

The Jackson Laboratory

## Our Mission



To strengthen and diversify the resources offered to the scientific community from JAX

- **Partner** with foundations, pharmaceutical and biotech companies, and other scientists worldwide to facilitate research into treatments of these less common diseases.
- **Engineer** new models and enhance existing models through genetic standardization and characterization to ensure reproducibility of data across labs over time.
- **Distribute** well-characterized, preclinical mouse models to accelerate drug discovery for rare and orphan diseases.
- **Establish** phenotypically relevant outcome measures for use in preclinical efficacy testing platforms

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## ALS: A Complex Disease

A progressive neurological disease that affects the control of muscle movement caused by damage to motor neurons

Incidence of ALS is two per 100,000 people, average age of onset is 55 years; survival 3-5 years after diagnosis

Several risk factors associated with ALS. Military veterans twice as likely to develop ALS. Neurotoxins, cyanobacteria, regional incidences…..

Majority of cases are considered sporadic, only ~10% familial

Tremendous heterogeneity in disease onset and progression, even within families carrying the same familial mutation

Riluzole and Radacava only approved drug with modest benefits and inconsistent results across patient population

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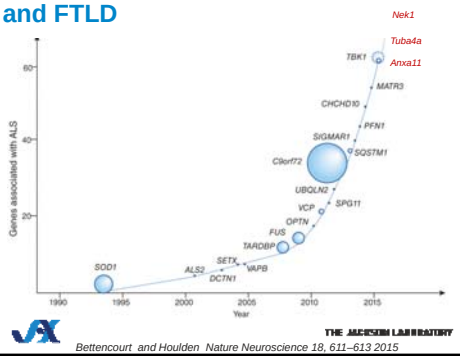
## Exome Sequencing Identifies New Genes Associated with ALS and FTLD

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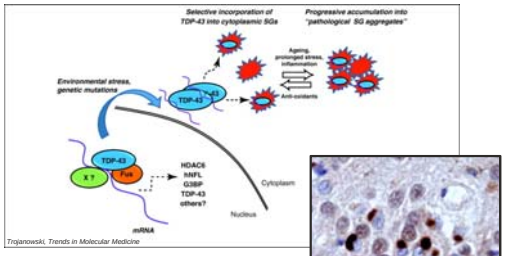
Bettencourt and Houlden Nature Neuroscience 18, 611–613 2015

## Revealing a Common Thread:

### 97% of Familial and Sporadic ALS Converge on TDP43 Pathology




Trojanowski, Trends in Molecular Medicine



Neumann, Int. J. Mol. Sci.

## MICE BUCKET CHALLENGE

Precision Genetics U54 ALS Disease Modeling Unit:

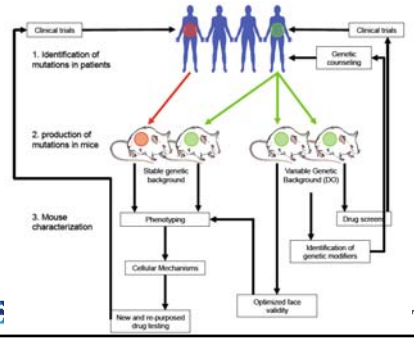
I. Identification of allelic variants that appear to cause ALS or contribute to susceptibility and/or severity of clinical presentation

II. *Creation of ALS-linked mutations in stable genetic backgrounds.*

III. *Create series of Prnp transgenic and inducible over-expressors*

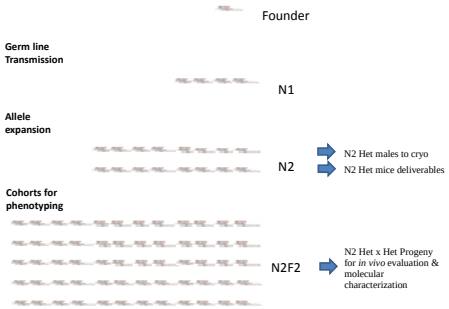
IV. *Exploring ALS Mutations in Collaborative Cross (CC) & Diversity Outbred (DO) mice*

## New ALS model development, preclinical testing and clinical applications




## The CRISPR Pipeline

Microinjection to N2F2 cohort distribution and analysis is 8.5 – 9.5 months




## New mutations made to date

Tbk1 R228H
Tbk1 N22D
Tbk1 G217R
Tbk1 del2

Tuba4a R320C
Tuba4a A383T
Tuba4a W407X
Tuba4a T145P
Tuba4a ins+1
Tuba4a del50

Tdp43 Floxed autoreg
Tdp43 G348C
Tdp43 A315T, M337V

Chchd10 S55L
Chchd10 R15L
Chcd10 del11
Chchd10 ins+1

B6NJ.Chchd10 R15L
B6NJ.Chchd10 S55L

Nek1 R261H
Nek1 del8
Nek1 R549X
Nek1 ex 2 -3 del

ANXA11_D40G
ANXA11_R235Q

Pfn C71G

Atxn2 ex 1 del

Fig4 I41T
Fig4 del11

Supplemental funding from:
- Muscular Dystrophy Association
- ALS Association
- Private Foundation

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## Expanding to Transgenics and Inducible Alleles

**Prion Promoter Driven:**

B6J.NEK1_WT_Prnp
B6J.NEK1_R540X_Prnp

B6J.TUBA4A_WT_Prnp
B6J.TUBA4A_T145P_Prnp
B6J.TUBA4A_R320C_Prnp

B6J.PFN1_WT_Prnp
B6J.PFN1_C71G_Prnp
B6J.PFN1_G118V_Prnp

B6J.TARDBP_WT_Prnp
B6J.TARDBP_K181E_Prnp

B6J.ANXA11_WT_Prnp
B6J.ANXA11_D40G_Prnp
B6J.ANXA11_R235Q_Prnp

B6J.ARPP21_WT_Prnp
B6J.ARPP21_P529L_Prnp
B6J.ARPP21_R713L_Prnp

B6J.TBK_WT_Prnp
B6J.TBK1_R228H_Prnp

**Tet Inducible:**

B6J.TBK_WT_TRE
B6J.TBK1_R228H_TRE

B6J.NEK1_WT_TRE
B6J.NEK1_R540X_TRE

B6J.TUBA4A_WT_TRE
B6J.TUBA4A_T145P_TRE
B6J.TUBA4A_R320C_TRE

B6J.PFN1_WT_TRE
B6J.PFN1_C71G_TRE
B6J.PFN1_G118V_TRE

Status of Transgenics
- Prion driven all made; assessing expression
- GLT established and mating for inducible

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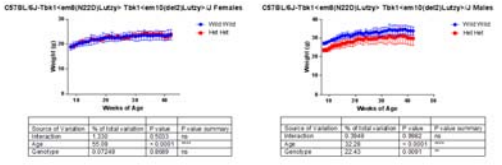
## Phenotyping Pipeline



- Bi-weekly Body Weight and Neurological scoring
- At 1 year of age:
  - Grip Strength
  - Gait analysis
  - Adhesive Removal Test
  - Erasmus Ladder
  - Histology

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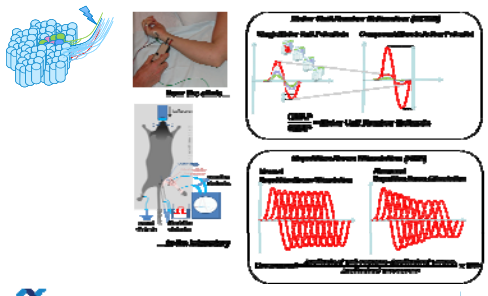
## Longitudinal Electrophysiology Assessment



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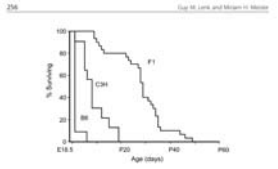
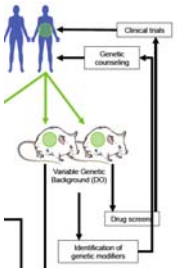
## The patient population is not a single haplotype but the B6 mouse is, so…….

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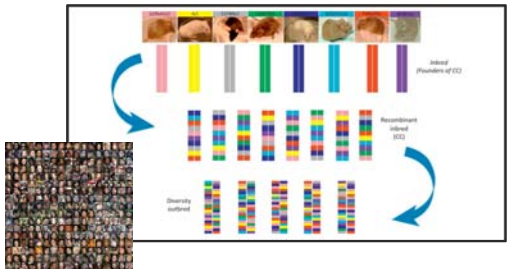
## Explore genetic heterogeneity in ALS



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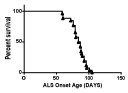
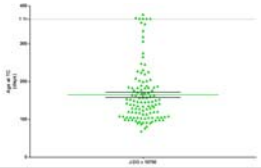
## Exploring Modifiers DO x Prp-TDP43 transgenic

B6. Tdp-43 Male survival




X

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## C9ORF72 AAV9 Induced Model

Science. 2015 Jun 5;348(6239):1151-4. doi: 10.1126/science.aaa9344. Epub 2015 May 14.

**Neurodegeneration. C9ORF72 repeat expansions in mice cause TDP-43 pathology, neuronal loss, and behavioral deficits.**

Chew J[1], Gendron TF[2], Prudencio M[2], Sasaguri H[2], Zhang YJ[2], Castanedes-Casey M[2], Lee CW[2], Jansen-West K[2], Kurti A[2], Murray ME[3], Bieniek KF[1], Bauer PO[2], Whitelaw EC[2], Rousseau L[2], Stankowski JN[2], Stetler C[2], Daughrity LM[2], Perkerson EA[2], Desaro P[2], Johnston A[2], Overstreet K[2], Edbauer D[4], Rademakers R[3], Boylan KB[3], Dickson DW[1], Fryer JD[1], Petrucelli L[2].

Diversity Outbred

X AAV9 C9Orf72
X AAV9 Tau

X 600

Scan with 150, 000 SNPs, RNA seq for expression QTLs

Will a percentage of mice develop paralysis? Will we see differential pathology? Can we map these modifiers, expression QTLs

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## What is a rare disease?


**WHO defines a rare disease as a disease that is debilitating or life threatening with a prevalence of 0.65% - 1% of the population.**

**50-75% of Rare disease affect children, and 30% die before 5.**

**Approximately 250 new rare diseases are described annually.**

**The combined number of individuals with rare diseases in the EU and US is estimated to exceed 55 million manifesting between 5,000 and 7,000 different rare diseases. Globally, the rare disease population is estimated at 350 million.**

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## Expanding JAX CRISPR Pipeline to Rare Diseases

Ngly1
Kif1a
Fxn
Psd
Trpv4
Sms
Ap4b1
Sumf1
Csnk2a1
Phip
Hecw2
Nexmif
Pacs1
Phps

## Summary

- *Working with ALS clinicians and researchers, we chose 9 new ALS genes to make precise humans mutations in corresponding mouse genes for a total of 28 new lines*
- *Secured additional funding to pursue transgenic lines, both Prion driven and inducible models*
- *Phenotyping pipeline has been established and most of the knock in models have been screened*
- *Initial experiments using genetic crosses to the DO mice have revealed potential for discovery of new modifiers*
- *Expanding CRIPSR pipeline at JAX to include models for Rare Diseases*