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

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

Our Mission

• 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.

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.
Exome Sequencing Identifies New Genes Associated with ALS and FTLD

Revealing a Common Thread: 97% of Familial and Sporadic ALS Converge on TDP43 Pathology

ICE 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

Supplemental funding from:
- Muscular Dystrophy Association
- ALS Association
- Private Foundation
Expanding to Transgenics and Inducible Alleles

Prion Promoter Driven:
- 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

Tet Inducible:
- B6J.TBK_WT_TRE
- B6J.TBK1_R228H_TRE
- 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

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

Phenotyping Pipeline

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

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

Explore genetic heterogeneity in ALS

Exploring Modifiers DO x Prp-TDP43 transgenic
C9ORF72 AAV9 Induced Model

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?

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.

Expanding JAX CRISPR Pipeline to Rare Diseases

Ngly1
Kif1a
Psd
Sms
Trp4
Aqp4
Sumf1
Csnk2a1
Phip
Hece2
Neumf
Pacs1
Phip
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 CRISPR pipeline at JAX to include models for Rare Diseases

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