Characterization and Suppression of *SOD1*-mediated ALS Phenotype in *Drosophila melanogaster* Knock-in Model

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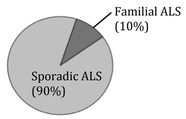
ABSTRACT

Amyotrophic Lateral Sclerosis (ALS) is the most common adult-onset neurodegenerative disorder, and is characterized by progressive motor neuron loss that results in muscle weakness, paralysis and eventually death. The molecular mechanisms underlying ALS remain largely unknown, in part, because most animal models of ALS fail to accurately mirror the disease. Whereas many labs have used transgenic overexpression models to produce an ALS phenotype, the Reenan lab has developed a knock-in genetic model in *Drosophila*. This model introduces specific mutations with known linkage to ALS in humans into the *Drosophila SOD1* gene locus. We observed a variety of disease phenotypes ranging in severity and onset, depending on the mutation introduced. This study seeks to further characterize the phenotypes of the various mutant lines. Imaging analysis of the most severe genotype, G85R/G85R, reveals a withered leg phenotype that may correlate to the muscle atrophy seen in human ALS. Additionally, while previous work has shown that H71Y/H71Y mutants are highly susceptible to the introduction of reactive oxygen species, the mutant lines included in the present study appeared to be only moderately susceptible to oxidative stress. We are currently performing a standard EMS mutagenesis suppressor screen. Notably, we have collected a number of surviving flies that appear healthy. However, further work is necessary to confirm the presence of a suppression mutation. We hope the completion of this screen will provide insight into the molecular mechanisms of this disease.

INTRODUCTION

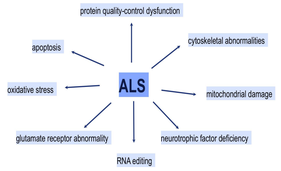
A + **myo** + **trophic** + **Lateral** + **Sclerosis**

absence + *muscle* + *nourishment* + *side (of the spine)* + *hardening*

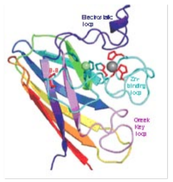


- ~80% motor neurons already dead at time of diagnosis [1]
- ~5,000 new cases/year in US
- 90% of cases sporadic; 10% of cases familial
- Average life expectancy of 3-5 years after diagnosis
- Only FDA-approved drug (Riluzole) extends life by only 3 months [2]

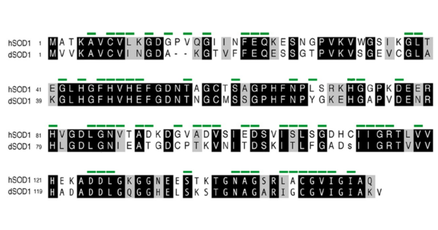
- *SOD1* first gene linked to ALS [2]
- Most genetic models have overexpressed mutant SOD1 at supraphysiological levels
- Reenan Lab uses ends-out homologous recombination (HR) to introduce specific *SOD1* mutations into the fly genome
  - Avoids pitfalls of transgenic overexpression
- Many proposed molecular mechanisms of disease



SUPEROXIDE DISMUTASE 1: Gene of Interest

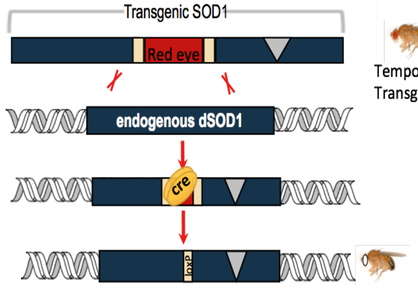


$O_2^- \leftrightarrows O_2 + H_2O_2$



- *SOD1* was the first gene linked to ALS
- Mutations in *SOD1* are reported in ~20% of familial and ~1.4% of sporadic (non-inherited) cases of ALS [2]
- Relatively short gene (~150 amino acids)
- Extensive homology between flies and humans

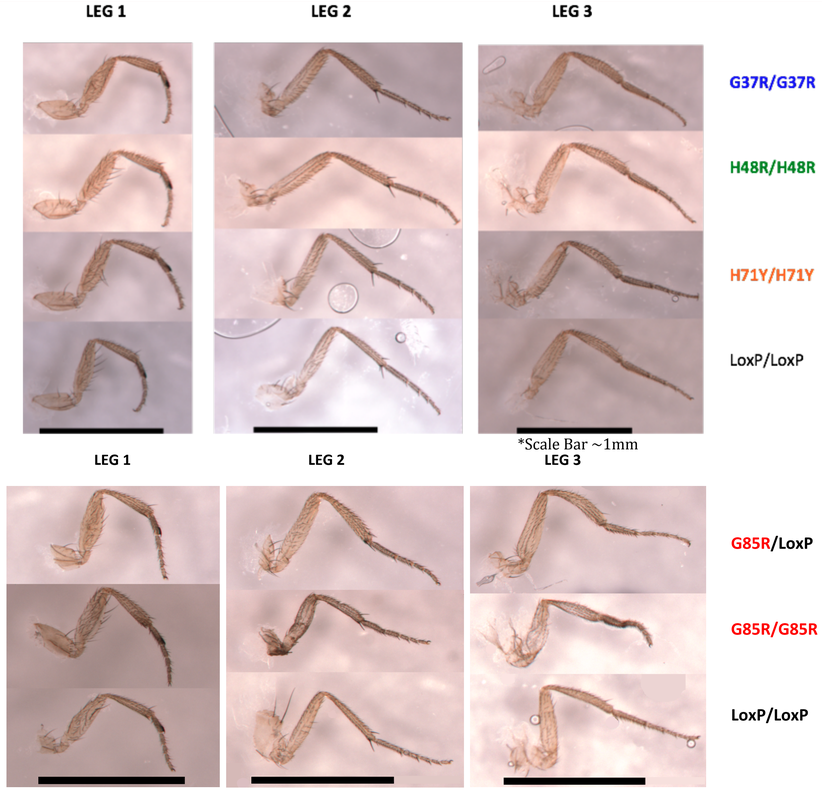
HOMOLOGOUS RECOMBINATION: Method of Choice



- Specific mutations introduced into *SOD1*
- Mutated form of the gene under same transcriptional regulation as control
  - Mutations not overexpressed
  - Endogenous protein not expressed
- Mutations vary in severity and onset of disease phenotype

G37R/G37R	H48R/H48R	H71Y/H71Y	G85R/G85R	LoxP/LoxP
Full Enzymatic Activity Early Onset	**Cu Binding Site Slow Disease Progression**	**Zn Binding Site Intermediate Severity Lives for 2 weeks**	**Enzymatically Inactive Most Severe Phenotype Dies at day 0**	**Full Enzymatic Activity Control**

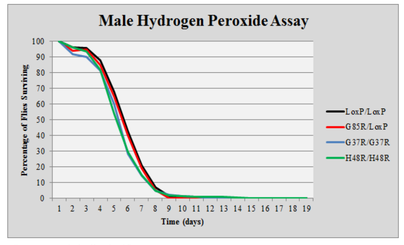
LEG IMAGING



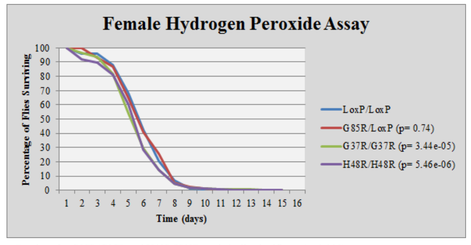
Representative images of *Drosophila* legs in mutant and control fly lines. Legs taken from young adult male flies.
Top Panel compares various SOD1 mutants against the LoxP/LoxP control. Little difference is seen in gross leg morphology.
Bottom Panel compares the G85R/LoxP and G85R/G85R mutants to the LoxP/LoxP control. G85R/G85R legs (especially leg 3) appear smaller and malformed.

HYDROGEN PEROXIDE (H_2O_2) ASSAY

Unlike G85R/G85R and H71Y/H71Y, some mutations do not have a phenotype reminiscent of ALS. Hydrogen peroxide was added to the diets of the organisms to determine whether oxidative stress induces an ALS-like phenotype in H48R/H48R and G37R/G37R mutants. Additionally, the hydrogen peroxide assay was conducted to confirm that G85R/LoxP flies are not highly susceptible to oxidative stress. Both male and female G85R/LoxP flies showed no statistically significant increase in sensitivity to hydrogen peroxide when compared to LoxP/LoxP controls. Female G37R/G37R and H48R/H48R flies displayed a statistically significant decrease in lifespan under oxidative stress, while males did not.

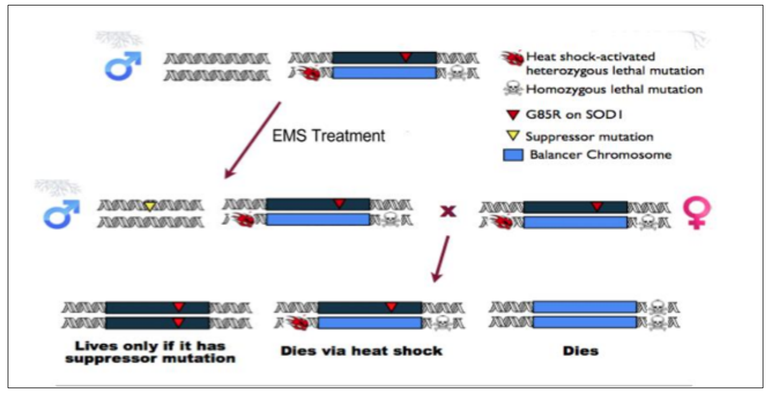


Data not statistically significant



P-values for G37R/G37R and H48R/H48R statistically significant ($p<0.005$)

EMS Mutagenesis Suppressor Screen



- Ethyl Methanesulfonate (EMS) is a mutagen often used on *Drosophila* models because of its high rate of mutation. EMS causes point mutations that can result in missense or nonsense substitutions.
- EMS suppressor screens with *Drosophila* models have been successful in the past [3].
- 4,000 G85R/balancer males were mutagenized and crossed with untreated virgin females to yield an F1 generation.
- Of the F1 generation, balancer/balancer (~25%) will not hatch, and G85R/balancer (~50%) will die after heat shock protocol due to activation of the *hs-hid* gene.
- G85R/G85R progeny of the F1 generation will only survive past 3-5 days if they have inherited a suppressor mutation from their mutagenized fathers that reverses or ameliorates the lethality of the ALS phenotype.
- Any fertile flies found with suppressor mutations (surviving longer than average G85R/G85R flies) will be back-crossed with heterozygous G85R flies in the hope of producing a line with a reversed ALS phenotype.
- The suppressor screen portion of this protocol is still in progress.
- The genomes of any suppressor flies will be sequenced and their relevant mutations characterized.
- Finding a suppressor mutation could lead to new research and potential new therapies for ALS patients.

DISCUSSION/FUTURE DIRECTIONS

- Significant leg deformities and muscle atrophy were observed in the G85R/G85R mutant flies; this observation supports the hypothesis that the G85R mutation is the most lethal.
- In other mutant lines, no significant changes in leg size/morphology were observed when compared to LoxP/LoxP controls.
- Future studies will use transcriptomic and bioinformatic analysis of gene expression profiles to determine the molecular factors that contribute to the lethality of the G85R mutation.
- The hydrogen peroxide assay suggests that the G85R heterozygotes are not significantly more sensitive to oxidative stress than the LoxP/LoxP controls; however, female G37R/G37R and H48R/H48R flies displayed a statistically significant decrease in lifespan while under oxidative stress.
- The EMS mutagenesis protocol is still in progress. Fertile flies surviving past five days will be back-crossed to the G85R/balancer line.
- The genomes of suppressor mutants will be sequenced and the suppressor mutations will be characterized.

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3. Brumby, A. "A Genetic Screen for Dominant Modifiers of a Cyclin E Hypomorphic Mutation Identifies Novel Regulators of S-Phase Entry in *Drosophila*." *Genetics* 168.1 (2004): 227-51. Web.

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