

Mutant SOD1 Transgenic Rodents as Models of FALS

- Transgenic mice and rats expressing mutant forms of SOD1 develop motor neuron pathology and clinical symptoms similar to those seen in patients with ALS.
- This affords researchers with excellent animal models of this disease.
- One of the most widely used animal models of ALS is the mouse expressing **multiple copies** of the G93A mutant form of the human SOD1 (hmSOD1) gene.
- In our experience, as well as that of other investigators, the clinical phenotype of these mice remains constant provided that the number of transgene copies is unchanged.

SOD1 Activity and ALS

- Measurements of SOD1 activity in blood from some patients with an SOD1 mutation indicated a loss of enzymatic activity. However, SOD1 activity in patients with other types of SOD1 mutations, such as G37R SOD1, retain full specific activity.
- Transgenic mice expressing different forms of mutant SOD1 develop progressive motor neuron disease despite markedly elevated SOD1 activity levels. The overall Cu/Zn SOD activity in these mice is elevated, not decreased, since each mouse possesses two normal, endogenous alleles plus the activity contributed by the mutant protein.
- Knock out mice in which SOD1 is completely deleted live normal life spans and do not develop overt motor neuron disease.
- In humans with familial ALS due to an SOD1 mutation, neither the age of onset nor rapidity of progression of disease correlate with dismutase activity levels.
- **In conclusion, most investigators feel, that the mutant enzymes have acquired one or more toxic properties, irrespective of the amount of SOD1 activity that each of them retains.**

High Expressor G93A SOD1 Transgenic Mice

- Our laboratory has established a colony of transgenic mice expressing the human gene for SOD1 with the G93A mutation.
- This colony was started from mice obtained from Jackson Laboratory line B6SJL-Tg(SOD1-G93A)1Gur.
- Our colony generally exhibits a phenotype typical of the Jackson Laboratories hSOD1Tg stock and is consistent with the predicted 25 copies of the SOD1 transgene (Dal Canto and Gurney, 1997).

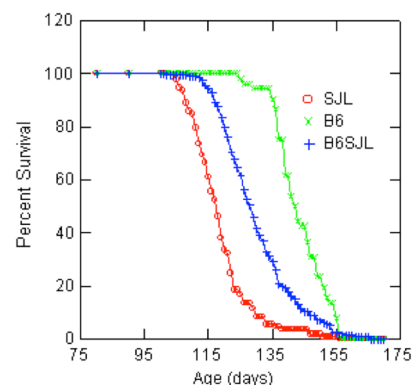
Proposed Mechanisms for Motor Neuron Loss in ALS tested in the hm SOD1 mouse

- **Oxidative Stress**
- **Glutamate-mediated excitotoxicity**
- **Formation of toxic intracellular aggregates** (of SOD1)/dysregulation of the proteasome
- **Neuroinflammation**

Variables in the Clinical Phenotype of the Drexel University School of Medicine G93A SOD1 Transgenic Mouse Colony¹

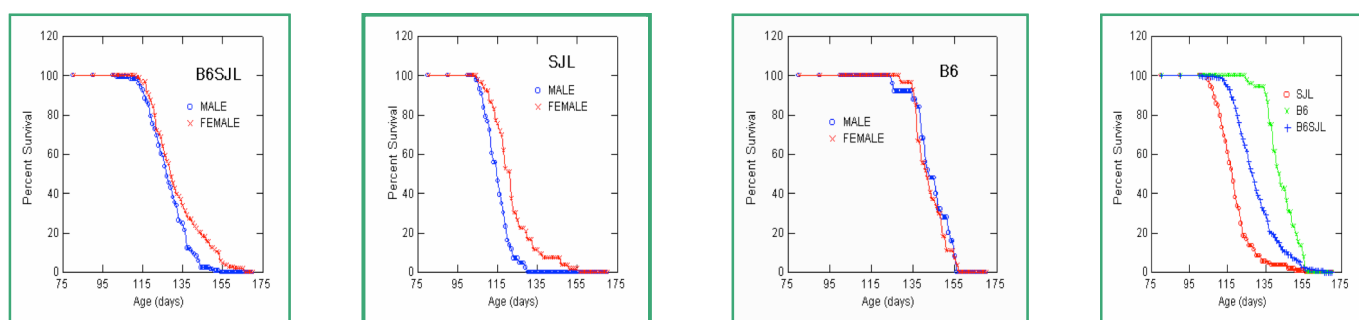
1. Genetic Background

The mean±SD survival of our (G93A)Tg⁺ mice in the B6/SJL background is **130.2 ±11.2 days** (n=224), range 102-166 days. The phenotype of the G93A SOD1 mutant mouse is dependent on genetic background.



2. Gender

In the B6/SJL colony, females survived significantly longer (132.8 + 12.4 days, n=103, range 112 - 166 days) than males (127.9+9.5 days, n=121, range 102-154 days) (p<0.001).

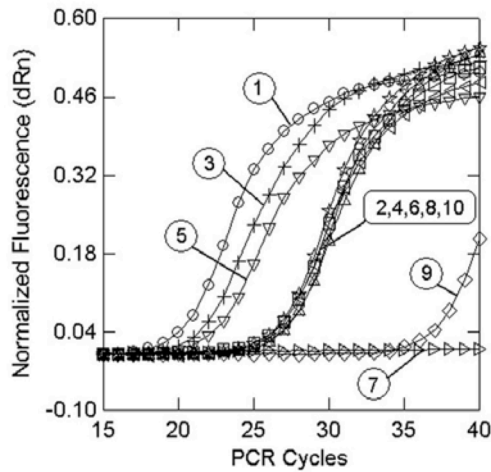


	(B6xSJL)Fn hSOD1 Tg ⁺			B6.Tg ⁺ Line			SJL.Tg ⁺ Line		
	Total	Males	Females	Total	Males	Females	Total	Males	Females
Number of Mice	224	121	103	52	25	27	96	43	53
Survival (mean ± SD) in days	130 ± 11	128 ± 9	133 ± 12	144 ± 8	144 ± 9	143 ± 7	119 ± 10	115 ± 8	123 ± 11

3. Transgene Copy Number

- Loss of transgene copies occur due to intralocus recombination events during meiosis.
- One recombination event occurred at Jackson Labs resulting in a transgenic line with a reduced number of copies (estimated as a drop from 25+1.5 to 8+1.5 copies) resulting in delayed disease onset and an increase in the mean life span from 132 to 251 days.
- Over the last four years, our colony has experienced four recombination events. All of the events resulted in mice demonstrating delayed onset of disease and an increase in survival.
- Although recombination events are rare, loss of transgene copies in a transgenic mouse used for breeding can devastate a small colony.

Using real time quantitative PCR, we have developed a method for determining changes in hSOD1 transgene copy number. [Alexander GM, et al. (2004) *Mol. Brain Research* **130**:7-15.]



		CT	Δ CT
A	1 ○ hSOD1 of B6S.JL-TgN(SOD1-G93A)1Gur	21.389	
	2 × mL2 of B6S.JL-TgN(SOD1-G93A)1Gur	28.491	7.102
B	3 + hSOD1 of B6S.JL-TgN(SOD1-G93A)dl1Gur	22.890	
	4 △ mL2 of B6S.JL-TgN(SOD1-G93A)dl1Gur	28.540	5.650
C	5 ▽ hSOD1 of B6S.JL-TgN(SOD1-G93A) DC#1	23.580	
	6 ◁ mL2 of B6S.JL-TgN(SOD1-G93A) DC#1	27.856	4.276
D	7 ▷ hSOD1 of B6S.JL-Tg-negative #1	> 40.0	
	8 □ mL2 of B6S.JL-Tg-negative #1	27.962	<-12.038
E	9 ◇ hSOD1 of B6S.JL-Tg-negative #2	38.558	
	10 ☆ mL2 of B6S.JL-Tg-negative #2	27.921	-10.637

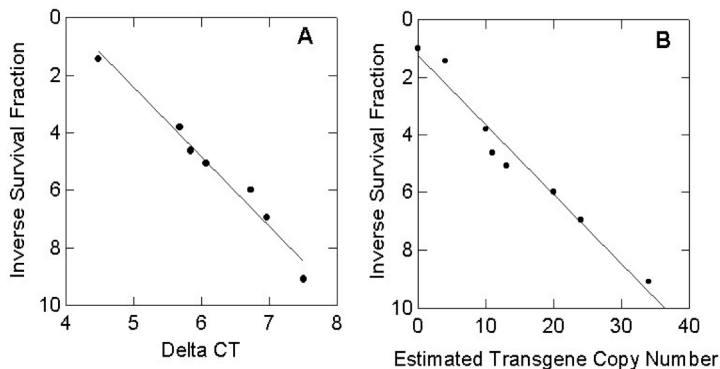
Real time normalized fluorescence amplification curves for human SOD1 and mouse IL2 from DNA of five mice (A-E). Three animals were SOD1 transgene positive (A, B and C) and two mice were transgene negative (D, E). There was no significant difference ($p > 0.05$) in mouse IL2 CTs between transgene positive and transgene negative mice (amplification curves 2,4,6,8 and 10). The amplification curves for human SOD1 in transgene positive mice (1,3 and 5) demonstrated CTs always less than the corresponding mL2 CT amplification curve. In contrast, the amplification curves for human SOD1 in transgene negative mice (7 and 9) demonstrated CTs always greater than the corresponding mL2 CT curve. The Δ CTs for transgene positive mice were always greater than zero and demonstrated higher values in mice with the more severe phenotypes ($A > B > C$).

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Survival vs. Transgene Copy Number in G93A SOD1 Mutant Mice

Mutant Type	Δ CT	Transgene Copies	Survival	SD	N
B6S.JL-HE _x LE	7.508	34	99		1
B6S.JL-HE	6.967	24	129.2	11.1	241
B6S.JL-LE _x LE	6.738	20	150.1	8.3	9
Drop Copy#3	6.074	13	174	14.8	20
Drop Copy#4	5.847	11	194.7	29.8	3
B6S.JL-LE	5.685	10	236.5	16.3	10
Drop Copy#2	5.376	8	na		
Drop Copy#1	4.484	4	625	60.8	5

(A) Relationship between the Inverse Survival Fraction (Survival of transgene negative B6/SJL mice* divided by the survival of transgene positive mice) and Δ CT ($r^2=0.966$, $p < 0.01$). (B) Relationship between the inverse survival fraction and the number of estimated mutant G93A human SOD1 transgene copies ($r^2=0.958$, $p < 0.01$). (*) The Jackson Laboratory has not gathered any statistics on the average life span of B6S.JL F1/J (Stock # 100012) F1 hybrid mice. For B6S.JL F1/J mice for which survival data has been collected, the mean life span is within the range of 850-950 days (personal communication from The Jackson Laboratory). In computing the inverse survival fraction, we choose a life span of 900 days for the non-tg animals.



G93A SOD1 Mouse Model of ALS: Use in Pre-Clinical Trials

Clinical Trials in the G93A Mouse: Measuring Time to Onset of Disease and Survival

Determination of End Stage (Survival)

- Natural death is not an end point. For humane reasons, progressive deterioration of the animal's health leading to death is not allowed.
- Survival is defined as the age at which the mouse demonstrates complete paralysis of one hind limb or is unable to right itself within 30 seconds of being placed on its side. At that time, the animal is sacrificed.

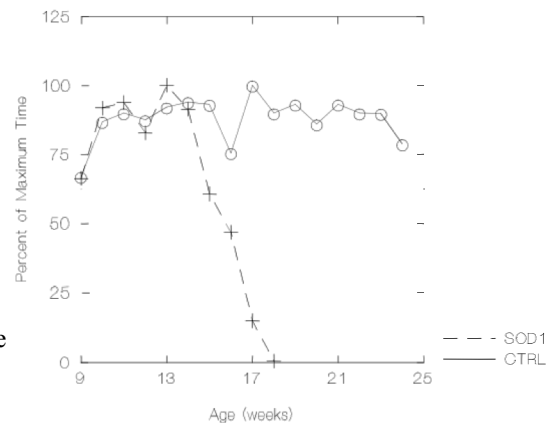


Determination of Onset of Clinical Symptoms

Onset of clinical symptoms is detectable by 70-90 days of age. These mice show muscle wasting, abnormal splay of the hind limbs and tremor.

A. Rotorod. We have used the rotorod to identify the time of onset of clinical signs.

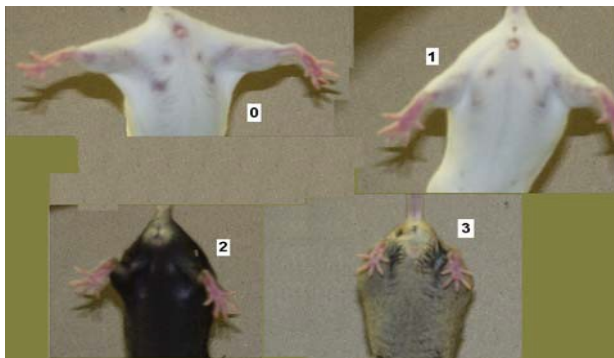
Rotorod data of controls (n=13) and SOD1 (n=15) animals starting at 60 days of age (Figure 1). For each animal 100% is taken as its maximum time in seconds that the animal remained on the rotorod. The rotorod was set at a constant rotation of 20 rpm, once the animal reached 5 minutes, it was removed from the apparatus and returned to its cage. Each animal was tested a minimum of two trials each week.



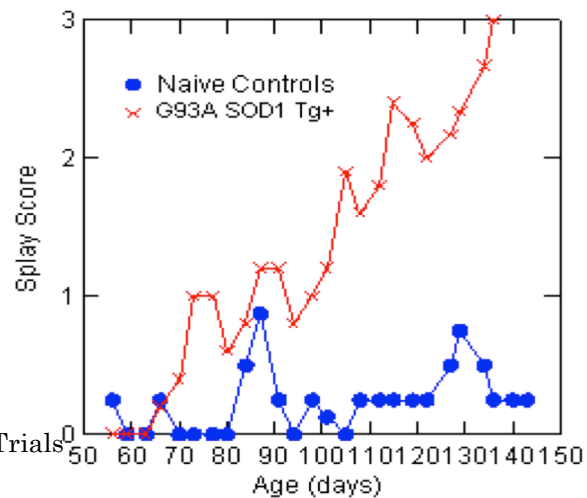
B. Combined score from three assessments:

1. Splay Test

(0=normal; 1=mild; 2=moderate; 3=severe)



Splay vs Age in Female B6/SJL mice



G93A SOD1 Mouse Model of ALS: Use in Pre-Clinical Trials

2. Beam Test

The mouse is placed on the edge of a plexiglas sheet (~0.3 inches wide) and observed while walking.

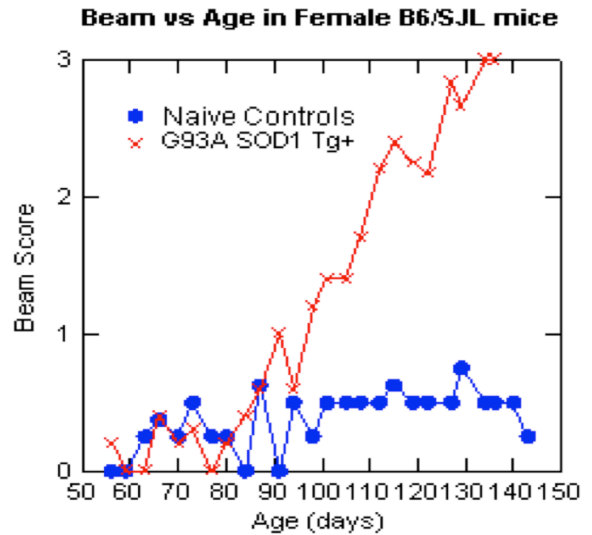
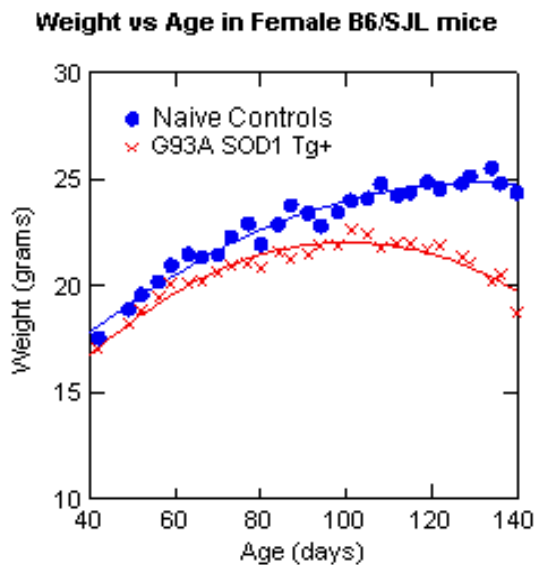
0 = Normal

1 = Mildly impaired (uses side of the cage)

2 = Moderately impaired (is barely able to walk)

3 = Severely impaired (is not able to walk and falls)

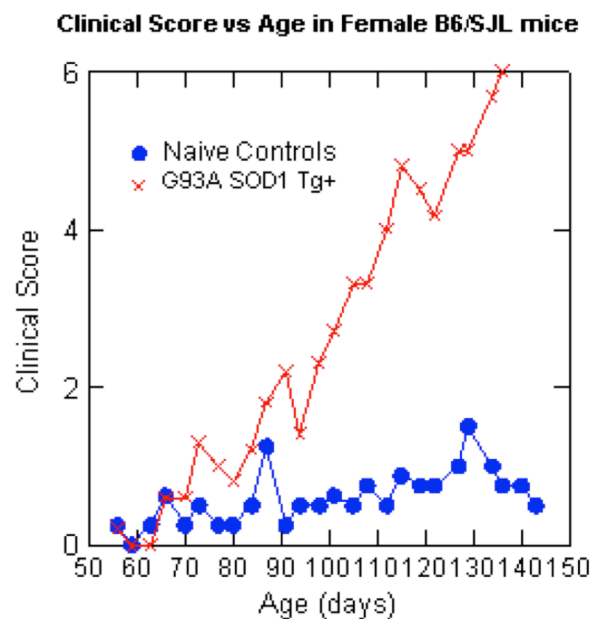
3. Weight



Example: Mildly impaired mouse (uses the side of the beam), giving a score = 1.

The Determination of Disease Onset

- Weight Onset was defined as the maximum of the weight data (zero of the first derivative) when fitted to a second order polynomial (parabola).
- Clinical onset was defined as the day that the sum of splay and beam test reached a value equal to 2 without going below 2.



¹References

Deitch, J.S., Alexander, G.M. Del Valle, L. and Heiman-Patterson, T.D. (2002) GLT-1 glutamate transporter levels are unchanged in mice expressing G93A human mutant SOD1. *J. Neurol. Sci.*, **193**:117-126.

Alexander, G.M., Erwin, K.L., Byers, N., Deitch, J.S., Augelli, B.J., Blankenhorn, E.P. and Heiman-Patterson, T.D. (2004) Effect of transgene copy number on survival in the G93A SOD1 transgenic mouse model of ALS. *Mol. Brain Res.*, 130:7-15.

Heiman-Patterson, T.D., Deitch, J.S., Blankenhorn, E.P., Erwin, K.L., Perreault, M.J., Alexander, B.K., Byers, N., Toman, I. and Alexander, G.M. (2005) Background and gender effects on survival in the TgN (SOD1-G93A)1Gur mouse model of ALS. *J. Neurol. Sci.*, **236**:1-7.

This model of ALS was developed by the ALS Research Group at the Drexel University College of Medicine and the ALS Hope Foundation, and this description of the model is copyrighted by the former.