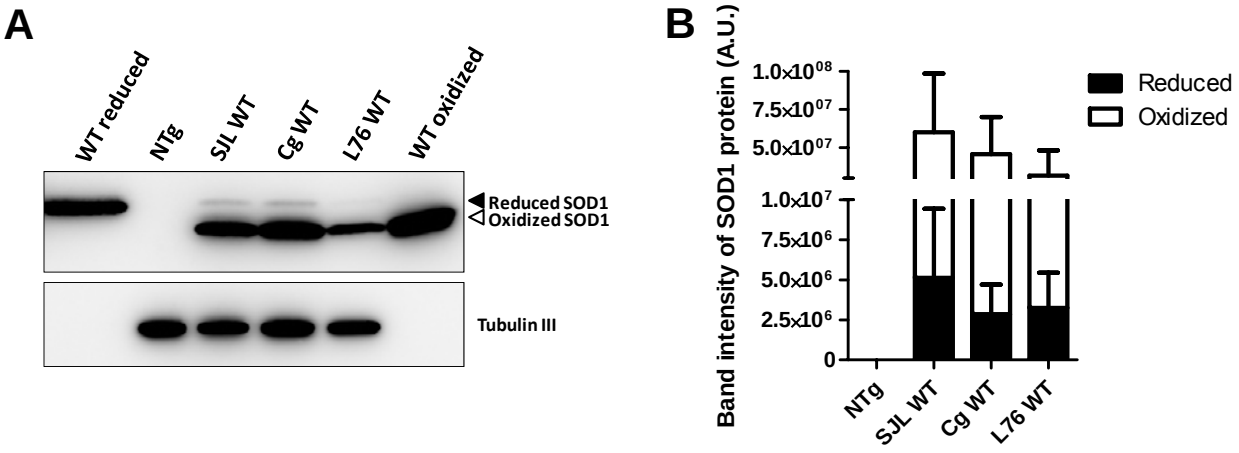
AN EXAMINATION OF WILD-TYPE SOD1 IN MODULATING THE TOXICITY AND AGGREGATION OF ALS-ASSOCIATED MUTANT SOD1

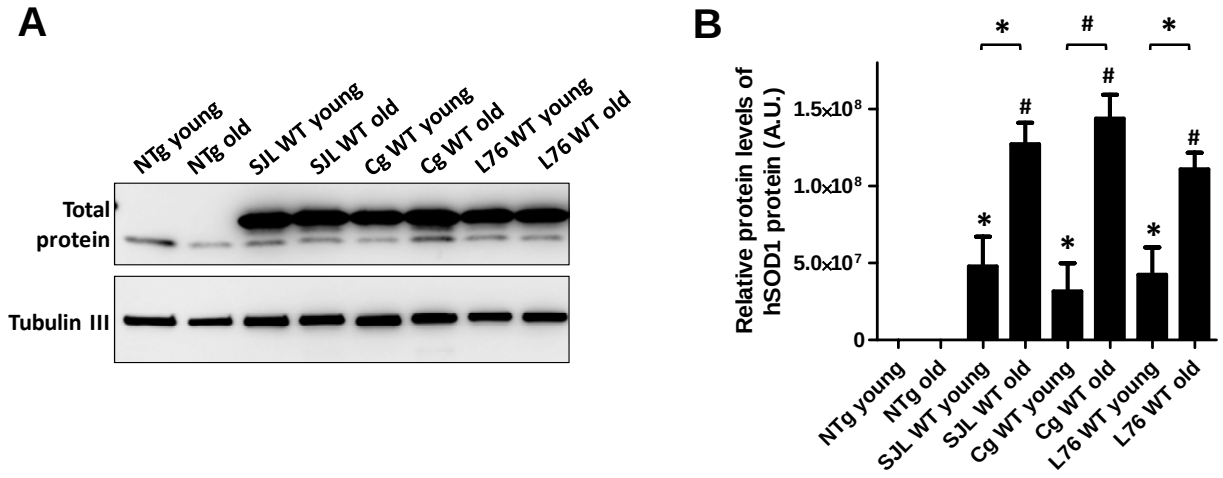
Prudencio et al. Supplementary information

Fig. S1.



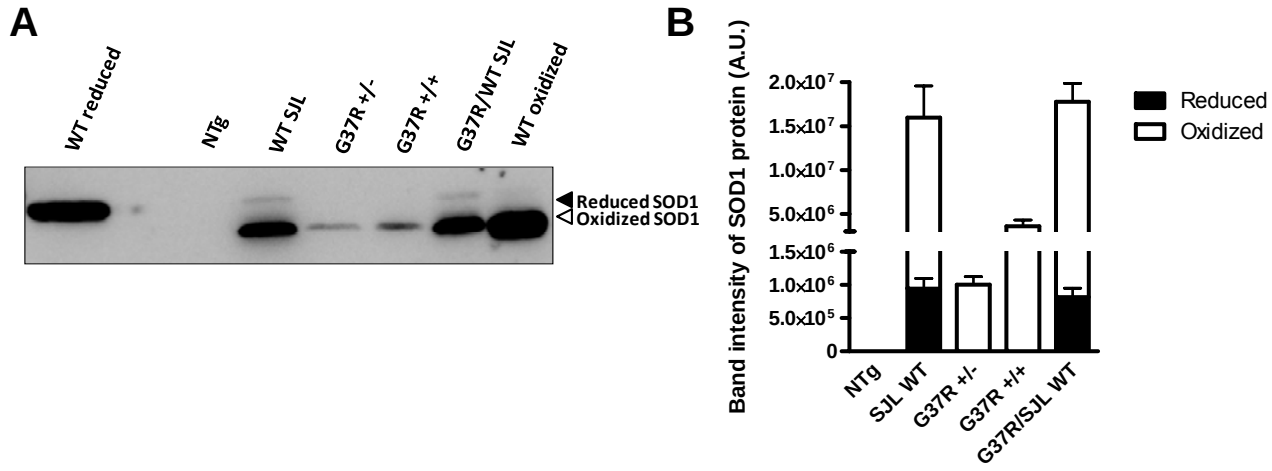
Low amounts of reduced WT hSOD1 protein are present in spinal cords of all WT lines of mice. A) Immunoblot of reduced and oxidized SOD1 proteins in spinal cord extracts of different lines of WT hSOD1 mice (> 11 months). β-tubulin III was used as a loading control. B) Quantification of the band intensities of at least three independent experiments to determine levels of reduced and oxidized WT hSOD1.

Fig. S2.



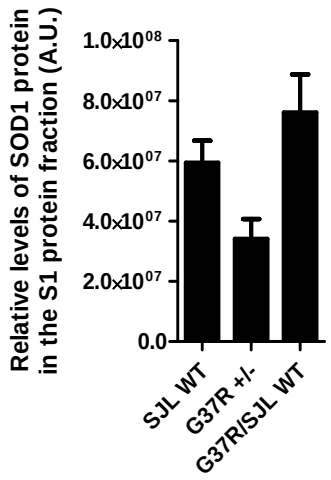
The levels of WT hSOD1 in spinal cords of transgenic mice increase with age. A) Immunoblot of total SOD1 levels in spinal cord from mice expressing WT hSOD1 at young (4 months for L76 and CgWT, or 8 months for SJLWT) or old (17 months for L76 and CgWT, or 11 months for SJLWT) ages. An antibody (m/hSOD1) that recognizes mouse (lower band, top panel) and human (upper band, top panel) SOD1 was used. NTg: non-transgenic mice. β-tubulin III was used as a loading control for total protein. B) Quantification of the total hSOD1 protein levels. Unpaired student *t*-tests were performed to establish significant differences with NTg mice, or as indicated: $^{*}p \leq 0.05$, $^{\#}p \leq 0.005$. Bars represent mean ± SEM.

Fig. S3



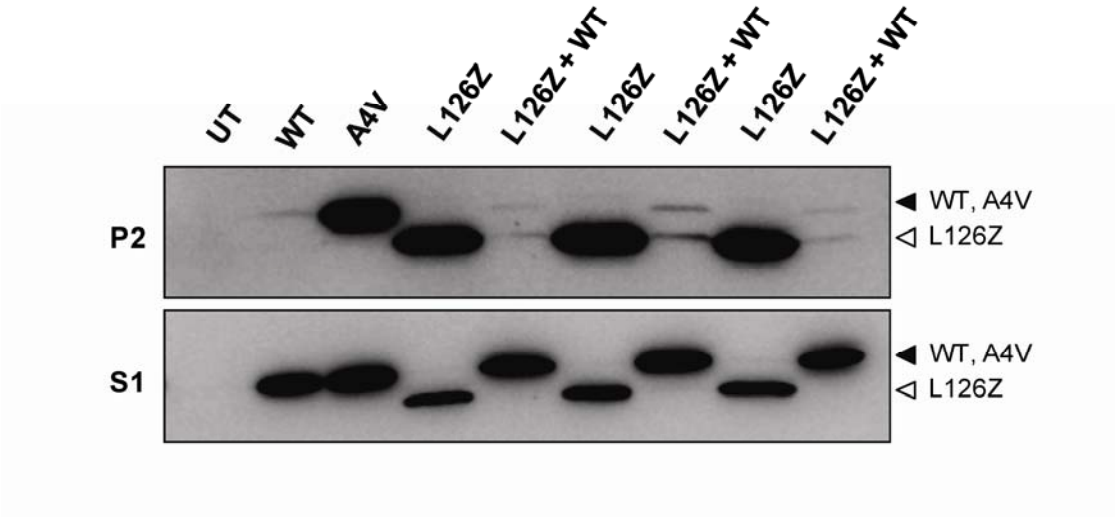
The majority of SOD1 in spinal cords of SJLWT, G37R, and G37R/SJLWT mice is normally modified by an intramolecular disulfide bond. A) Immunoblot of reduced and oxidized SOD1 proteins in spinal cord extracts of different lines SOD1 transgenic mouse lines (8 month old). To visualize reduced and oxidized forms of SOD1, samples were run on SDS-PAGE gels in the absence of reducing agent (β-mercaptoethanol, β-ME). Transferred membranes are briefly incubated in β-ME to allow better antibody binding, as described in (1). As controls, WT reduced and oxidized forms of recombinant SOD1 protein were prepared through treatment with or without β-ME, respectively. B) Quantification of the band intensities of at least 3 independent experiments from reduced and oxidized SOD1 proteins.

Fig. S4



SOD1 levels in the detergent-soluble (S1) fraction of spinal cords of heterozygous SJL WT and G37R mice, and G37R/SJLWT mice. The amount of SOD1 in the S1 fraction was calculated from western blot data. An example of the source of these data is presented in Fig.4A of the text. The level of total soluble hSOD1 in spinal cords of G37R+/- mice was about 50% lower than that of heterozygous SJLWT mice. As predicted, the levels of total soluble hSOD1 protein in spinal cords of G37R/SJLWT mice were higher than that of the SJLWT mice.

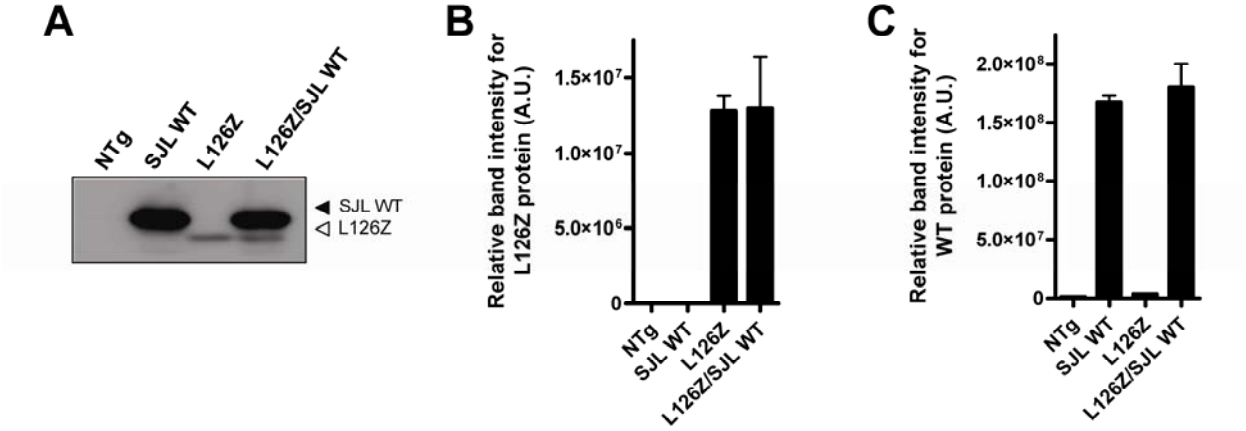
Fig. S5



Immunoblots of detergent-insoluble and soluble fractions from HEK293FT cells transfected with expression vectors for mutant and WT SOD1. Cells were transfected with vectors for WT, A4V, L126Z, and L126Z with WT. After 48 hours, the cells were then fractionated into detergent soluble and insoluble fractions. This blot analyzes 3 separate transfection experiments for L126Z and L126Z+WT. In cells transfected with L126Z+WT, very low amounts of insoluble WT and L126Z protein were detected whereas in the soluble fraction we only detected the WT protein. The same plasmid DNA preparations used for the L126Z transfections was used in the mixed L126Z+WT transfections. Each lane contains either 5 (S1) or 20 (P2) μg protein. The blot was probed with hSOD1 antibody, which specifically recognizes human SOD1.

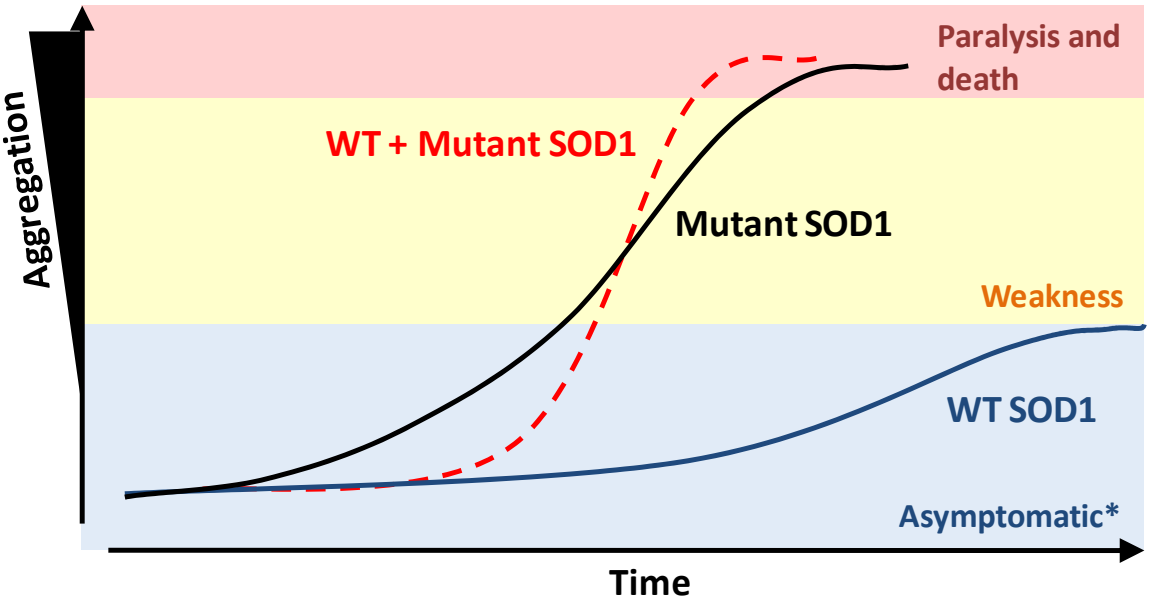
The lack of accumulation of L126Z protein in the soluble fractions of the L126Z+WT transfection is probably due to the rapid turnover of this mutant. When aggregation of the L126Z mutant is inhibited by the presence of the WT protein, then virtually none of this mutant accumulates in either the soluble or insoluble fractions.

Fig. S6



Immunoblots of total forebrain protein, comparing heterozygous SJLWT mice to heterozygous L126Z mice and L126Z/SJLWT doubly transgenic mice, demonstrates no obvious change in steady-state levels of L126Z mutant protein in the presence of high levels of WT protein. A) Immunoblots of total forebrain protein from nontransgenic, SJLWT, L126Z, and L126Z/SJLWT mice. Each lane contains 10 μg of total protein. Blots were probed with hSOD1 antibody, which specifically recognizes human SOD1. B) Quantification of L126Z protein in mice of all four genotypes (n=3 animals of each genotype). C) Quantification of WT SOD1 in mice of each genotype (n=3 animals of each genotype).

Fig. S7.



Hypothetical model on the effect of WT hSOD1 on disease and aggregation in mice expressing mutant hSOD1. The asterisk (*) indicates mice that exhibit no obvious symptoms of weakness.

In the standard model of protein aggregation there are 3 steps: 1) A nucleation phase, in which initial subunits of misfolded SOD1 come together to form a “core” from which aggregation initiates; 2) A growth phase, where detergent insoluble species accumulate exponentially; and 3) A final phase, in which the supply of monomeric species is exhausted. The onset of visible signs of weakness in the mouse models coincides with the beginning of the growth phase of aggregate formation (1). Aggregation of mutant hSOD1 occurs at a rate that varies depending on the SOD1 mutation (2). Aggregation of WT hSOD1 occurs at much lower rates, with detectable accumulation of detergent-insoluble species when expressed for long periods of time and at very high levels. Finally, in the co-expression of WT and mutant hSOD1, we propose that the nucleation phase of aggregation would be lengthened because our data indicate that the presence of WT hSOD1 slows the aggregation of mutant hSOD1. It is possible that interactions between mutant and WT SOD1 proteins lessen the chance of mutant proteins to interact in ways that promote aggregation. It is also possible that nucleation of WT-mutant complexes occurs more slowly. Ultimately, once nucleation of mutant hSOD1 proteins occurs, the growth phase could be faster as both mutant and WT proteins are captured in aggregates.

Reference List

1. Karch,C.M., Prudencio,M., Winkler,D.D., Hart,P.J., and Borchelt,D.R. (2009) Role of mutant SOD1 disulfide oxidation and aggregation in the pathogenesis of familial ALS. Proc.Natl.Acad.Sci.U.S.A., 106,7774-7779.

2. Prudencio,M., Hart,P.J., Borchelt,D.R., and Andersen,P.M. (2009) Variation in aggregation propensities among ALS-associated variants of SOD1: correlation to human disease. Hum.Mol.Genet., 18,3217-3226.