Amyotrophic Lateral Sclerosis (ALS) is a common neuromuscular disease without effective treatment characterized by the selective degeneration of motor neurons resulting in paralytic death. In a familial subset of ALS, mutant Cu/Zn superoxide dismutase (SOD1) enzymes initiate and sustain this disorder dose-dependently. SOD1 is a 153-residue β-barrel homodimer that serves as the predominant cytosolic scavenger of neurotoxic superoxide anion radical (O₂⁻). Determining the gain-of-function mechanism of SOD1 toxicity for therapeutic intervention in ALS has been the subject of continuing research for over fifteen years.

Pathogenic SOD1 species aberrantly oligomerize, and a portion of these multimers form cytoplasmic mass aggregates.

Collection of soluble and insoluble mutant SOD1 for in-gel reduction

- Every toxic SOD1 mutant published thus far forms distinct but relatively variable detergent-soluble aggregates whereas wildtype protein (WT) and other innocuous SOD1s remain persistently soluble (1 and references therein).
- Relative to a mutant standard, mutant aggregation potential in HEK cell culture is correlate with a shorter disease duration in patient cohorts (1).
- Spinal cord extracts of symptomatic transgenic mice show rapid accumulation of aggregate SOD1 over the course of disease (2), and deposition occurs near-selectively in the ventral horn (3).

**CONCLUSIONS**

Both D101N and D101G form significant detergent-insoluble aggregates in HEK cell culture, although D101N nucleation is delayed. D101N&G carriers have just 2.5 years average time from diagnosis to death. D101N&G have similar rates of deposition and a similar threshold but dissimilar nucleation, indicating metrics that may be more correlative with clinical data. This is not the case for all variable mutants at one position. More time course and pulse chase experiments are underway to test this hypothesis. The D101G lesion results in a loss of dismutase activity (and intraduced protein), whereas the D101N mutant is electrophoretically and enzymatically WT-like. Notably, the apparent trimers and pentamers are resistant to 5% ME and appear in both the S1 and P2 fractions, as do E133V’s -ME resistant oxidized disulfides. E133V retains no aggregate intraduced protein even in extended cell culture. The E133V-FFP fusion protein is comparable to WT-FFP in that neither aggregate in adherent cells even in extended cell culture. SOD1s extracted in the presence of NP-40 and EDTA have diminished activity and tightened bands relative to TN extracts (data not shown). Thus SI assays are an underrepresentation of actual activity. However, it does not appear the degree to which detergents or chelators effect SOD1 proteins vary significantly interspecfically. Although it is unlikely soluble SOD1s are active, the detergent extract design (rines and SDS/deoxycholate) is not suitable for enzymatic analysis.