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# SOD1 prions transmit templated aggregation and fatal ALS-like disease

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## Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av filosofie/medicine doktorsexamen framläggs till offentligt försvar i E04, R-1, Norrlands Universitetssjukhus, byggnad 6E, fredagen den 11 October, kl. 13:00.
Avhandlingen kommer att försvaras på engelska.

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## Abstract
Amyotrophic lateral sclerosis (ALS) is an adult-onset fatal neurodegenerative disease characterized by a progressive degeneration of the upper and lower motor neurons. The resulting paresis begins focally, usually in one muscle, and spreads contiguously, leading to muscle wasting, progressive paralysis and eventually death. 90% of all ALS cases are sporadic, with no genetic background (sALS), while 10% are hereditary or familial (fALS). The first identified cause of ALS was mutations in the gene encoding the enzyme superoxide dismutase 1 (SOD1), which are found in 3-6% of the ALS patients. Mutations in SOD1 confer a cytotoxic gain of function on the enzyme. Cytosolic inclusions containing aggregated SOD1 in motor neurons are a hallmark of ALS, both in patients and transgenic (Tg) mice carrying mutant human SOD1s (hSOD1). These inclusions have also been reported in sporadic and familial ALS cases without SOD1 mutations, suggesting a broader role of this protein in the ALS pathology. However, the mechanism of SOD1 misfolding and aggregation, and their contribution to the disease pathogenesis, is unclear.
Our research group has recently identified two structurally different strains of hSOD1 aggregates (denoted A and B) in the central nervous system of Tg murine models expressing full-length hSOD1 variants.
The aim of this thesis is to investigate if the SOD1 aggregation is a collateral byproduct in the process of the disease, or if it drives ALS pathogenesis. In addition, this work investigates the spreading characteristic of the disease *in vivo*.
Human SOD1 A and B seeds were prepared from spinal cords of terminally ill hSOD1 Tg mice by ultracentrifugation through a density gradient. Minute amounts of the aggregate seeds were micro-inoculated into the lumbar spinal cord of asymptomatic recipient Tg mice, overexpressing G85R mutant hSOD1 ($hSOD1^{G85R}$). Mice inoculated with A or B aggregates developed early-onset fatal ALS-like disease, becoming terminally ill around 100 days after inoculation. This is nearly 200 days earlier than $hSOD1^{G85R}$ Tg mice inoculated with a control preparation or non-inoculated mice. Concomitantly, exponentially growing templated hSOD1 aggregation developed in the recipient mice, spreading all along the neuraxis. The pathology provoked by the A and B strains differed in aggregation growth rates, disease progression rates, aggregate distribution along the neuraxis, rates of weight loss, end-stage amounts of aggregates, and histopathology.
Next, we explored the existence of mutant hSOD1 aggregates with prion-like properties in the spinal cord of ALS patients. To this end, aggregate seeds were prepared from the spinal cord of the autopsy material of an ALS patient carrying the $hSOD1^{G127X}$ truncation mutation, as well as from mice transgenic for the same mutation. The aggregates showed a strain A-like core structure. Inoculation of both the murine and human derived seeds into the lumbar spinal cord of hSOD1 expressing mice efficiently transmitted strain A aggregation, propagating rostrally throughout the neuraxis and causing premature fatal ALS-like disease. The inoculation of human or murine control seeds had no effect. The potency of the ALS patient-derived seed was exceedingly high, and the disease was initiated under conditions plausible to exist also in the human motor system. These results demonstrate for the first time, the presence of hSOD1 aggregates with prion-like properties in human ALS.
We extended the exploration of hSOD1 prion mechanisms by inoculating another recipient mouse line, with wild-type-like stability and essentially normal SOD activity. Mice that are hemizygous for the $hSOD1^{D90A}$ transgene insertion do not develop ALS pathology and have normal murine lifespans (>700 days). Homozygous mice develop ALS-like disease around 400 days-of-age. Interestingly, inoculations of both strain A and B seeds into the lumbar spinal cord of hemizygous $hSOD1^{D90A}$ mice induced progressive hSOD1 aggregations and premature fatal ALS-like disease after around 250 and 350 days, respectively. In contrast, hemizygous $hSOD1^{D90A}$ mice inoculated with a mouse control seed died from senescence-related causes at ages beyond 700 days.
Altogether, data in this thesis shows that the hSOD1 aggregate strains are ALS transmitting prions, suggesting that prion-like growth and spread of hSOD1 aggregation is the core pathogenic mechanism of SOD1-induced ALS.

## Keywords
ALS, amyotrophic lateral sclerosis, SOD1, prion, neurodegeneration, strain, seeding, protein misfolding, protein aggregation, propagation, transgenic mice

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