SOD, ORF and ALS: 
On the role of \textit{SOD1} and \textit{C9ORF72} 
in the pathogenesis of ALS

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Abstract
Amyotrophic lateral sclerosis (ALS) is characterized by adult-onset degeneration of upper and lower motor neurons. Symptoms begin focally in one muscle and then spread contiguously, resulting in progressive paralysis and death from respiratory failure. Hexanucleotide repeat expansion in C9ORF72 is the most common genetic cause, however, mutations in SOD1 were the first identified and are found in 1-9% of patients. Misfolded SOD1 aggregates in the CNS are hallmarks of ALS associated with SOD1 mutations. However, accumulation of misfolded or aggregated SOD1 protein has also been reported in sporadic and familial ALS without SOD1 mutations, suggesting that wild-type SOD1 could play a role in ALS pathology in general.

The aims of this thesis are: 1) To describe the resulting disease phenotype and specific characteristics of the SOD1 protein carrying the stable disease-associated mutation L117V. 2) To set up cell-based in vitro models to study the mechanisms of SOD1 misfolding and aggregation under physiologically relevant expression levels. 3) To compare SOD1 activity in patient-derived samples and screen for underlying causes of deviant SOD1 activities in individuals lacking SOD1 mutations.

1) We identified a novel L117V SOD1 mutant in two families of Syrian origin that co-segregated with the disease. This mutation was associated with slow disease progression, reduced penetrance and a uniform phenotype. The L117V mutant protein was indistinguishable from wild-type SOD1 in terms of stability, dismutation activity and misfolding in patient-derived cell lines.

2) We established patient-derived fibroblast and iPSC-MN lines expressing mutant SOD1 at physiological levels as in vitro models to study misfolding and aggregation of SOD1. We investigated the effects of several cellular pathway disturbances on SOD1 misfolding. Misfolded SOD1 was increased by inhibition of the ubiquitin-proteasome pathway in fibroblasts derived from both patients and controls. An age-related decline in proteasome activity could contribute to the late onset of ALS.

Next, we studied the effects of low oxygen tension on misfolding and aggregation of SOD1 in patient-derived cells. Low O2 tensions were found to markedly increase C57-C146 disulphide reduction, misfolding and aggregation of SOD1. Importantly, the largest effects were detected in iPSC-MNs. This suggests that motor neurons are specifically vulnerable to misfolding and aggregation of SOD1 under low O2 tension.

3) We compared the enzymatic activity of SOD1 in blood samples from a large number of ALS patients and controls. We screened for potential underlying causes of deviant SOD1 activities in individuals lacking SOD1 mutations. No aberrations in copy number, other large structural changes in introns and exons or intronic mutations in the 30-50 bp flanking the exons were found in the 142 outliers, with either very low or very high SOD1 dismutation activities. However, hemoglobinopathies, including thalassemias and iron deficiency anemia, were associated with high SOD1/mg Hb ratios. Erythrocytes from patients with destabilizing SOD1 mutations showed half the normal activity. There were no significant differences in SOD1 activity between control individuals and ALS patients without a coding SOD1 mutation, or carriers of TBK1 mutations or the hexanucleotide repeat expansion in C9ORF72. Our result suggests that SOD1 enzymatic activity is not associated with the disease in non-SOD1 mutation ALS.

Keywords: ALS, SOD1, misfolded species, proteasome, low oxygen tension, induced pluripotent stem cells-derived motor neurons, enzymatic activity, erythrocytes