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IVV-13 Beneficial Effect of Oxaloacetate for the Neuromuscular Function of SOD1G93A Mice

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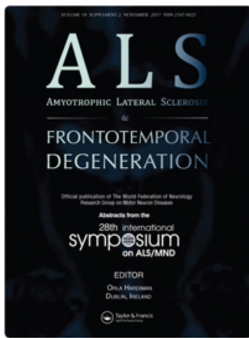

Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration

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Theme 3 *In Vivo* Experimental Models

IVV-01 Increased aggregated SOD1 in spinal cord from SOD1(G93A) transgenic mice correlates with later disease onset and improved longevity

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Keywords: SOD1, aggregation, spinal cord

Background: Superoxide dismutase (SOD1)-positive aggregates have been detected in the spinal cords and brains of people living with amyotrophic lateral sclerosis caused by mutations in SOD1. Transgenic mice over-expressing mutant human SOD1 harbor similar SOD1-positive inclusions. It remains unclear what role these SOD1 inclusions play in ALS disease pathogenesis. It has been proposed that SOD1 aggregation drives disease in familial ALS. Indeed, mutant SOD1 variants with higher *in vitro* propensities to aggregate seem to correlate with more aggressive ALS clinical variants. While mutant SOD1 variant instability and aggregation propensities may influence disease onset and progression, it remains unclear whether the aggregation process *in vivo* accelerates or slows neurodegeneration. The two possibilities are not mutually exclusive.

Objectives: To determine whether increased SOD1 aggregate load in spinal cord correlates with accelerated disease progression in SOD1(G93A) mice.

Methods: B6SJL-TGN(SOD1-G93A)1Gur mice were assigned to the study at p50 ($n = 30$, 15 male/15 female), assessed for neurological disease progression and body weight daily, and designated for terminal tissue collection based on three specific stages of clinical disease presentation regardless of age. 10 mice were sacrificed when the animals first showed signs of paresis, 10 were sacrificed when animals first showed complete paralysis in their hind limbs, and 10 mice were sacrificed when the mice were moribund as a function of ALS-like disease. Relative aggregate load in spinal cord lysate from each mouse was assessed using a 0.2 micron methylcellulose filter retardation assay system with luminescent detection of SOD1 immunoreactivity.

Results: Relative SOD1 aggregate load in SOD1(G93A) transgenic mice increased with age. However, aggregate load was not strictly associated with disease stage. Younger moribund mice carried lower aggregate loads than older moribund mice ($R^2 = 0.6492$). Younger paralyzed mice carried lower aggregate load than their older counterparts ($R^2 = 0.9689$). Younger mice at onset of paresis carried lower aggregate loads than their older counterparts at similar stages of disease progression ($R^2 = 0.2081$). Female mice carried higher aggregate loads than male mice regardless of disease stage ($p<0.02$).

Discussion and conclusions: These data do not support the conclusion that SOD1 aggregates markedly accelerate ALS disease progression *in vivo*. The data support the hypothesis that SOD1 aggregation may be a neutral, age-dependent phenomenon in SOD1(G93A) mice. The data may also support the hypothesis that processes that govern development of SOD1-positive aggregates larger than 0.2 microns are protective in SOD1(G93A) mice. Increased aggregate load correlated with later onset of each disease stage characterized. It is plausible that SOD1 aggregation *in vivo*, results in sequestration of deleterious SOD1 species or other molecules responsible for more aggressive disease phenotypes. These results suggest that therapeutic approaches, geared solely toward reducing aggregate loads in the spinal cord of SOD1(G93A) mice, may not improve disease outcomes.

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IVV-02 Misfolded SOD1 levels in the blood of SOD1(G93A) transgenic mice as indicators of ALS disease progression

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Keywords: SOD1, ERAD, blood

Background: In patients with SOD1 mutations, the central nervous system (CNS) has been shown to be enriched for misfolded mutant SOD1, especially in degenerating motor neurons. Mutant transgenic ALS mouse models have also been shown to have lifelong enrichment of several species of misfolded SOD1 in their CNS parenchyma. The CNS parenchyma is suboptimal for tissue biopsy in humans; however, if misfolded SOD1 could be detected in the blood, it has the potential to be used as a biomarker for humans living with ALS.

Objectives: To determine whether misfolded SOD1 is detectable in the blood compartment of SOD1(G93A) mice and to understand biological processes regulating its levels.

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Methods: To investigate misfolded SOD1 protein levels, B6SJL-TGN(SOD1-G93A)1Gur mice were bled using repeated mandibular bleeds from day 60 to moribund (end-stage ALS). During this time, scores for neurological disease progression (NeuroScore) and body weight were captured daily. Misfolded SOD1 in whole blood and bone marrow was detected with ELISA using the NI204.B anti-SOD1 antibody from Neurimmune. To investigate mRNA expression in bone marrow, SOD1 mice were scored and weighed daily starting at age 35 days. The mice were sacrificed at first instances of scores indicating paresis, paralysis, and moribundity. Bone marrow was collected and RNA was isolated. Low-density array cards were designed with genes from pathways relating to endoplasmic reticulum-associated protein degradation (ERAD), ER chaperones, apoptosis, and autophagy. RT-qPCR was run on the array cards and Ct values were normalized using geNorm.

Results: Misfolded SOD1 is detectable in blood and bone marrow, and its concentration decreases significantly as disease severity increases. The amount of misfolded SOD1 in the blood of pre-symptomatic male mice is inversely correlated with disease onset and age at death ($p = 0.01$). RT-qPCR analysis of mRNA expression in bone marrow indicates statistically significant up-regulation of ERAD genes, with no corresponding up-regulation of genes involved with ER chaperones, apoptosis, or autophagy ($n = 84$) ($p < 0.05$).

Discussion and conclusions: The ability to consistently detect misfolded SOD1 in blood and the inverse correlation between levels in blood and disease severity in mice suggest that misfolded SOD1 is a potential prognostic indicator of disease progression in this mouse model. Up-regulation of ERAD genes in bone marrow, and therefore likely in RBC (red blood cell) precursors, suggests that pathways in the blood compartment compensate in response to the presence of misfolded SOD1.

Acknowledgments: The anti-misfolded SOD1 antibody was provided by Neurimmune.

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IVV-03 Pharmacological inhibition or genetic ablation of complement C5a receptor, C5aR1, ameliorates disease pathology in the hSOD1^{G93A} mouse model of amyotrophic lateral sclerosis

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Keywords: neuroinflammation, disease progression, mouse

Background: Amyotrophic lateral sclerosis (ALS) is a fatal and rapidly progressing motor neuron disease without effective treatment. The complement system is up-regulated in ALS, with recent studies indicating that the activation product C5a may accelerate disease progression via its receptor, C5aR1 (1–3).

Objective: This study examined the pathological role of C5aR1 in hSOD1^{G93A} mice, using a combination of C5aR1 knockout mice, and pharmacological inhibition.

Methods: The selective and orally active C5aR1 antagonist, PMX205, was administered to hSOD1^{G93A} mice via their drinking water, both pre- and post-disease onset. Blood, brain and spinal cord pharmacokinetics were performed using LC-MS/MS methods. C5aR1$^{-/-}$ mice were also backcrossed to hSOD1^{G93A} mice to generate hSOD1^{G93A} mice deficient in C5aR1 (hSOD1^{G93A} × C5aR1$^{-/-}$) and were compared alongside hSOD1^{G93A} mice. The effect of C5aR1 genetic ablation and PMX205 treatment on disease progression of hSOD1^{G93A} mice was determined using body weight, hind limb grip strength, survival time and blood analysis.

Results: PMX205 entered the intact central nervous system at pharmacologically active concentrations, with increased entry observed in hSOD1^{G93A} mice as disease progressed, in line with augmented blood-brain-barrier breakdown ($n = 4$, $p < 0.05$). hSOD1^{G93A} mice treated with PMX205 prior to disease onset, or hSOD1^{G93A} × C5aR1$^{-/-}$ mice, both had significantly improved hind-limb grip strengths, slower disease progression and extended survival, compared with vehicle treated or control hSOD1^{G93A} mice ($n = 13$, $p < 0.05$, PMX205; $n = 15$, $p < 0.01$, hSOD1^{G93A} × C5aR$^{-/-}$). These improvements in the PMX205-treated group were associated with reductions in pro-inflammatory monocytes and granulocytes, and increases in T-helper lymphocytes in the peripheral blood ($n = 6$, $p < 0.05$). Importantly, PMX205 treatment beginning several weeks following disease onset also had an attenuating effect on disease progression, significantly extending survival ($n = 9$, $p < 0.05$).

Discussion and conclusions: These results confirm that C5aR1 plays a pathogenic role in hSOD1^{G93A} mice, further validating the C5a-C5aR1 signaling axis as a potential therapeutic target to slow disease progression in ALS.

Acknowledgements: This work was supported by the Motor Neurone Disease Research Institute of Australia (MNDRIA) through MNDRIA Grant-in-aid and post-doctoral fellowship. This work was also supported by a National Health and Medical Research Council project grant (APP1082271) and a Career Development Fellowship (APP1105420).

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IVV-04 Ablation of free fatty acid receptor 2 (FFAR2) signaling accelerates early disease progression in the SOD1^{G93A} mouse model of amyotrophic lateral sclerosis

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Keywords: neuroinflammation, FFAR2, mouse

Background: The gut microbiome and short chain fatty acids (SCFAs) have been shown to modulate inflammatory responses in both the CNS and peripheral tissues (1,2). Specifically, SCFA signaling through the SCFA-receptor, FFAR2, has been shown to influence systemic inflammation (3), as well as microglia morphology and activation (2). Chronic neuroinflammation plays a pathological role in ALS (4), and ALS patients and ALS mice show microbiome dysbiosis (5,6), suggesting that FFAR2 could play a role in ALS disease progression.

Objective: This study aimed to determine the function of FFAR2 in ALS disease progression by comparing SOD1^{G93A} mice to SOD1^{G93A} mice deficient in FFAR2 (SOD1^{G93A} × FFAR2$^{-/-}$).

Methods: Neuromotor assessments were conducted weekly beginning at 42 days to identify differences in motor symptoms (motor score and hind-limb grip strength), along with body weight and survival ($n = 13$–15). Neuroinflammation was measured at disease end stage by determining the degree of gliosis and cytokine expression using quantitative PCR ($n = 6$).

Results: There was no difference in the survival of SOD1^{G93A} × FFAR2$^{-/-}$ mice compared to SOD1^{G93A} mice. However, SOD1^{G93A} × FFAR2$^{-/-}$ mice demonstrated reduced body weight throughout disease progression relative to SOD1^{G93A} mice ($p<0.0001$). SOD1^{G93A} × FFAR2$^{-/-}$ mice also displayed greater neuromotor dysfunction at an earlier age, compared to SOD1^{G93A} mice with worsened motor scores (49 days vs. 77 days; $p<0.0001$) and reduction in hind-limb grip strength ($p<0.0001$). Surprisingly, and in contrast to the worsened disease progression, there was reduced gene expression for gliosis markers (CD11b, Iba-1 and GFAP) and pro-inflammatory cytokines (TNFα and IL-1β) in SOD1^{G93A} × FFAR2$^{-/-}$ mice, relative to SOD1^{G93A} mice, at disease end stage ($p<0.05$, $p<0.01$, $p<0.0001$).

Discussion and conclusions: Overall, these results indicate that FFAR2 may play a protective role in the early stages of disease progression in the SOD1^{G93A} mice, but switches to a pathological impact at later stages of the disease. Additional quantitative PCR analysis of gliosis and inflammation at earlier stages of the disease could reveal the potential dual role of FFAR2 on neuroinflammation and pathology throughout the disease progression of ALS.

Acknowledgements: This study was supported by NHMRC project grant (APP1082271).

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IVV-05 Fibroblast growth factor-2 (FGF-2)-dependent interplay of neurotrophic factors and signalling cascades in amyotrophic lateral sclerosis

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Keywords: growth factors, signaling cascades, FGF-2

Background: Neurotrophic growth factors have been described as key players in the development, function and maintenance of motor neurons and are thus implied in the pathogenesis of amyotrophic lateral sclerosis (ALS). We previously described that the knockout of fibroblast growth factor-2 (FGF-2), in the SOD1G93A transgenic mouse model of ALS, results in a prolonged survival attributed to a delay in disease onset. These neuroprotective effects are, at least, partially associated with compensatory up-regulation of other growth factors (CNTF and GDNF) (1).

Objectives: Our goal was to characterize the expression patterns and the protein levels of FGF-2 and its dependent growth factors, as well as growth-factor activated signaling cascades in ALS transgenic mice. Moreover, we aimed to better understand the impact of FGF-2 and its isoforms as paracrine factors on the cellular level.

Methods: Gene expression and protein analyses were performed to determine the levels of growth factors and signalling molecules at different time-points, in order to elucidate their roles during disease progression. For this purpose, spinal cord and muscle homogenates of SOD1 (B6.Cg-Tg (SOD1*G93A) 1Gur/J) and wild-type, as well

as double mutant (FGF-2 deficient SOD1*G93A transgenic) mice were analyzed at day 90, 120 and 150. To examine the effects of FGF-2 in neural cell-cell interactions, primary mouse motor neurons with two distinct ALS mutations (SOD1, TDP43) or wild-type, respectively, were co-cultured with cortical astrocytes of different FGF-2 genotypes (wild-type, hetero- or homozygous knockout).

Results: Growth factors and several players of respective signalling cascades were significantly up- or down-regulated, in both spinal cord and muscle samples, in a disease stage-dependent manner. *In vitro,* both wild-type and SOD1, motor neurons show a significant increase in motor neuron number when co-cultured with homozygous FGF-2 knockout astrocytes. These results imply a role of FGF-2 in motor neuron survival and degeneration.

Discussion and conclusions: Detailed characterization of neurotrophic factor spatiotemporal expression patterns, including cell specific analyses of motor neurons and glial cells, as well as the evaluation of the dependent signalling pathways, will further elucidate disease-specific dysregulations and their therapeutic relevance.

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IVV-06 The influence of neurotrophic factors on *in vivo* axonal transport in the SOD1^{G93A} mouse

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Keywords: in vivo axonal transport, neurotrophic factors, SOD1G93A

Background: Axonal transport is vital for maintaining neuronal function and survival through the bi-directional movement of cargoes, such as proteins, organelles and mRNAs, between neuronal soma and distal compartments. Given its significance on neuronal homeostasis, it is unsurprising that defective axonal transport is associated to amyotrophic lateral sclerosis (ALS) (1). Deficits in axonal transport are observed pre-symptomatically in the SOD1^{G93A} mouse, suggesting that impaired transport contributes to pathology (2). Rescuing motor neuron axonal transport dynamics may ameliorate pathology and extend survival (3,4). Neurotrophic factors (NTFs) mediate survival through local and distal signalling via axonal transport and are attractive treatment options aimed at restoring axonal transport dynamics. Motor neurons respond differently to individual NTFs and a greater pro-survival effect is observed when treated in combination (5). However, the influence that NTFs have on *in vivo* axonal transport dynamics throughout disease progression is currently unknown.

Objectives: (1) Determine the influence of NTFs on *in vivo* axonal transport dynamics; (2) Assess whether muscle fibre type influences axonal transport dynamics; (3) Characterise the longitudinal effects of NTFs in SOD1^{G93A} mice.

Methods: To image *in vivo* axonal transport, wild-type and SOD1^{G93A} mice were injected in the tibialis anterior or gastrocnemius muscle with 5 μg of fluorescently-labelled atoxic fragment of tetanus neurotoxin (H_cT), with either 25 ng of brain-derived neurotrophic factor (BDNF), 25 ng of glial-derived neurotrophic factor (GDNF), or phosphate buffered saline (PBS). 4–61 hours later, sciatic nerves were exposed in live, anaesthetized animals, and imaged using confocal laser scanning microscopy at 37 °C. Time-lapse microscopy was performed on sciatic nerve axon bundles to assess axonal retrograde transport dynamics of H_cT-labelled signalling endosomes using the TrackMate plugin (FIJI).

Results: In wild-type mice, signalling endosome velocities were greater in BDNF- and GDNF-treated cohorts compared to PBS controls. Signalling endosomes paused more frequently and for longer in the PBS-treated cohort. In the SOD1^{G93A} mouse, the treatment trends continued with axonal transport dynamics slowing down during disease progression. The longitudinal analysis of NTFs in disease progression will be discussed during the presentation.

Discussion and conclusions: We show that exogenous application of NTFs influences axonal transport dynamics, suggesting that NTFs elicit signals by binding to their membrane receptors, which enhances the rate of transport of signalling endosomes. This effect may be due to the direct modulation of motor complex activity and/or an increase in their recruitment on signalling endosomes and has implications in the discovery of novel treatment strategies.

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IVV-07 Muscle type specific abnormalities in terminal Schwann cell morphology following partial denervation in the SOD1 G93A mouse

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Keywords: collateral reinnervation, terminal Schwann cells, muscle fiber phenotype

Background: Muscles become increasingly denervated as motor axons withdraw from motor endplates and eventually die in ALS. Motor axons sequentially withdraw from fast contracting Type IIb muscle fibers, later from Type IIa and lastly from slow contracting Type I fibers. This progressive loss of motor innervation results in increasing levels of partial denervation. Typically, denervated endplates in partially denervated muscles are reinnervated by axonal sprouts extending from neighboring motor neurons. This process is known as collateral reinnervaton and is mediated, in part, by terminal Schwann cells (TSCs) (1). Collateral reinnervation is compromised, however, in ALS. This lack of plasticity contributes to the rate of muscle paralysis and disease progression (2).

Objectives: To determine whether muscle-fiber-type susceptibility to denervation in ALS contributes to impaired collateral reinnervation.

Methods: We partially denervated (PD) the soleus (composed of Type IIa and Type I fibers) and plantaris (composed of Type IIa and Type IIb fibers) muscles in $SOD1^{G93A}$ mice and WT littermates by unilaterally cutting the 5th lumbar spinal nerve prior to motor neuron cell death (ie P30). Mice recovered for 14–30 days to determine innervation recovery, or for 1–3 days to examine TSC morphology in response to denervation. Plantaris and soleus muscles from both PD and unoperated legs were then removed, fixed, and immunostained in whole mount preparations of teased muscle fiber bundles. Endplate innervation was quantified by comparing colocalization of pre-(Tuj1/SV2) and post-synaptic (α-bungarotoxin) structures, while TSC presence was determined using a Schwann cell-specific marker (S100β).

Results: PD plantaris muscles contained significantly fewer innervated endplates compared to PD soleus muscles in $SOD1^{G93A}$ mice ($p = 0.0143$), and their counterparts in WT mice ($p = 0.0079$) ($n = 5$), 14 days after nerve transection. Unlike PD muscles in WT mice and PD soleus muscles in $SOD1^{G93A}$ mice, the number of TSCs associated with denervated endplates in PD plantaris muscles in $SOD1^{G93A}$ mice decreased over time. This decrease was significantly different 3 days after nerve transection ($p<0.001$, $n = 4$). There was no evidence of activated caspase-3 (ie cell death) at any of the denervated endplates examined. Finally, denervated endplates without TSCs were always associated with Type IIb muscle fibers ($p = 0.0143$) ($n = 4$).

Discussion and conclusions: Lack of collateral sprouting and reinnervation of denervated Type IIb fibers in PD plantaris, but not the soleus muscle in $SOD1^{G93A}$ mice explains, in part, why the two muscles are differentially affected in ALS. Studies are currently underway to determine why TSCs do not remain at these endplates following PD in $SOD1^{G93A}$ mice.

Acknowledgements: Funding from ALS Canada, Canadian Institute of Health Research, Natural Sciences and Engineering Research Council of Canada, and the Killam Trusts.

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IVV-08 Astrocyte-derived extracellular vesicles contribute to the propagation of pathogenic proteins in ALS

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Keywords: extracellular vesicles, SOD1, astrocytes

Accumulation of pathogenic proteins is a major hallmark of neurodegenerative diseases including amyotrophic lateral sclerosis (ALS), and a propagation of pathogenic proteins is implicated in the disease progression. Recent studies indicate that extracellular vesicles (EVs) such as exosomes contribute to the propagation of pathogenic proteins. However, the mechanisms underlying protein propagation in ALS remain unclear. We investigated a role for astrocyte-derived exosomes in protein propagation in ALS. The levels for exosome-related molecules, such as neutral sphingomyelinase 2 (nSMase2) and CD63 were up-regulated in activated astrocytes during disease progression of $SOD1^{G93A}$ mice. In addition, p62-positive inclusions were prominently observed within spinal astrocytes in symptomatic $SOD1^{G93A}$ mice, suggesting defective autophagy in $SOD1^{G93A}$ astrocytes. Inhibiting autophagy promoted secretion of mutant SOD1-bearing EVs from $SOD1^{G93A}$-primary astrocytes. Moreover, administration of EVs derived from AcGFP-$SOD1^{G93A}$-expressing T98G glioma cells resulted in the accumulation of mutant SOD1 protein in NSC-34 cells. Furthermore, astrocyte-specific deletion of mutant SOD1 from $loxSOD1^{G37R}$ mice, which slowed the disease progression of ALS, resulted in a significant reduction of mutant SOD1 in spinal motor neurons. These findings suggest that autophagy dysfunction in astrocytes contributes to the propagation of mutant SOD1 by promoting secretion of EVs and that astrocyte-derived EVs might be a therapeutic target of ALS.

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IVV-09 Innate immune adaptor TRIF slows disease progression of ALS mice by eliminating aberrantly activated astrocytes

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Keywords: neuroinflammation, astrocyte, SOD1

Background: Recent evidence suggests that not only immune reactions, but also glia-immune interactions, contribute to disease processes of neurodegenerative diseases. Although the adaptive immune system was shown to be involved in the disease process of amyotrophic lateral sclerosis (ALS), the role of the innate immune system in ALS was not clarified. We aim to clarify the roles of Toll-like receptor (TLR) signaling in ALS model mice.

Methods: $SOD1^{G93A}$ mice (mSOD1) were crossbred with TRIF-deficient- and MyD88-deficient mice. Analyses for survival times, neuropathology, and mRNA levels for glia-related molecules were performed.

Results: TRIF-deficient mSOD1 mice exhibited an acceleration of disease progression by 50%, with shorter survival times by 24 days; however, MyD88 deficiency showed no significant effect on the survival times of mSOD1 mice. Although TRIF-deficient mSOD1 mice expressed lower levels of chemokines with fewer infiltrating immune cells in the spinal cord, these immune cells marginally affected disease course by eliminating lymphocytes (NK cells and CD8 T-cells) using the administration of antibodies. On the other hand, we found that aberrantly activated astrocytes were accumulated in TRIF-deficient mSOD1 spinal cords during disease progression. These astrocytes expressed both GFAP and Mac-2, and are positive for apoptotic markers and p62.

Discussion and conclusion: Despite dominant roles of MyD88 in TLR signaling, our results indicated TRIF-dependent TLR signaling was determinant for disease progression. Contrary to expectation, the dominant population of infiltrating lymphocytes had marginal effect in disease course. On the other hand, our data suggest that the TRIF pathway plays an important role in protecting the brain environment by eliminating aberrantly activated astrocytes through the cell-autonomous apoptotic pathway.

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IVV-10 The NF-κB signaling pathway is activated by converging microglial mechanisms in an ALS mouse model

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Keywords: microglia, proteome, inflammation

Background: Amyotrophic lateral sclerosis (ALS) is a non-cell-autonomous disease (1), meaning that not only neurons but other cell types are involved in the neuropathogenesis; cell types such as oligodendrocytes, astrocytes or microglia. Our study focuses on the latter. Microglia is known to acquire a pro-inflammatory phenotype with the progression of ALS. A recent study has shown that, in ALS, microglia can induce motor neuron death through the activation of NF-κB signaling pathway (2).

Objective: With this project, we aim to understand the molecular mechanisms of ALS in microglia by studying its proteomic signature in mice lumbar spinal cords. Then we want to understand how certain proteins implicated in the NF-κB signaling pathway are differentially regulated throughout the disease.

Methods: To do so, we generated and characterized a mouse model for cell-type specific microglial molecular profiling. This mouse expresses a fusion protein, mRPL10a-eGFP-Flag under the monocytic promoter CD11b. We crossbred this Ribotag mouse with the ALS mouse model expressing $hSOD1^{G93A}$. This model was used to immunoprecipitate microglial ribosomes and peptides in translation. Peptides were eluted and sequenced through mass spectrometry. Results were analyzed on the application ClueGO on the program Cytoscape to generate interactomes and study the regulation of proteins and pathways. The implication of target proteins was studied *in vitro* to elucidate their role on the NF-κB signaling pathway modulation.

Results: Through proteomic analysis, we were able to identify the proteins implicated in the NF-κB signaling pathway. We principally targeted two proteins differentially regulated between the presymptomatic and symptomatic stage of the disease, G3bp2 and Hspb1. Their regulation patterns suggest an activation of the NF-κB signaling pathway in the later stage. G3bp2 can bind IκBα/NF-κB complexes to keep them in the cytoplasm. Knocking down that gene results in a NF-κB activation in BV2 cells. Hsbp1, on the other hand, helps IKK phosphorylate IκBα, leading to the proteasomal degradation of the latter. Knocking down Hspb1 leads to an inactivation of the NF-κB signaling pathway.

Conclusion: The up-regulation of Hspb1 and down-regulation of G3bp2 at the symptomatic stage of the disease are two converging microglial cellular mechanisms to activation of the NF-κB signaling pathway. We suggest that by studying and modulating the regulation of microglial proteins linked to inflammation in ALS, we should be able to change the course of the disease. Our mouse model will allow us to identify possible treatment targets and

biomarkers to study the progression of the disease, both much needed in the field of amyotrophic lateral sclerosis.

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IVV-11 A unique subpopulation of astroglia regulate dendritic spines and growth and are lost during ALS disease progression in murine and human iPS models

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Keywords: astroglia, subpopulations

Introduction: Astroglia are the most abundant glia cell type in the CNS. Astroglia play crucial roles in maintaining homeostasis and assisting in processes such as neurotransmitter recycling, generation and elimination of neuronal synapses, controlled release of neurotrophins, and maintenance of the blood-brain barrier. Historically, astroglia have been placed into two groups: fibrous astroglia of the white matter, and protoplasmic astroglia of the gray matter. With our recent studies and those of others, it is becoming increasingly apparent that astroglia diversity goes beyond simple morphological depictions. For example, we now know that in development there is a great deal of astroglial heterogeneity and different populations of spinal cord astroglia can be identified. However, little is understood regarding the biological, physiological and molecular identity of adult astroglia in the cortex and spinal cord.

Methods and results: We employed multiple different TdTom-promoter reporter mice for the astroglial specific protein EAAT2, to identify cortical astroglial subtypes and define their molecular and physiological properties, along with their alterations in ALS disease models, as well as human control and ALS (C9orf72/mutant SOD1) iPS derived astroglia. By employing Tg mice expressing different lengths of the EAAT2 promoter (from 1.5->12 kb) we were able to identify unique regions responsible for astroglial specific expression. More importantly and unexpectedly, these tools elucidated a unique population layer 5 and 2/3 astroglia along with ventral spinal cord gray matter astroglia, with molecular properties remarkably different from other cortical and subcortical astroglia. Following FAC sorting and RNA seq/proteome analyses, a number of genes were found to be selectively expressed 30- to >1000-fold in these astroglia, including Kir4.1, LGR6 and others. With knowledge of these genes and their proteins, similar astroglia were identified in the human motor cortex and human control and ALS iPS astroglia. Selected genes from these astroglia were shown, *in vitro* and *in vivo*, to powerfully regulate motor cortex layer 5 neuronal dendritic growth, arborization and spine formation. Given the association with cortical motor neurons, we also investigated the alteration in these astroglia in ALS rodent models. We observed that these astroglia (both cortical and spinal cord) were dramatically affected in the ALS rodent model. By the end stage, this astroglia population is almost completely lost in areas of motor neuron degeneration (eg the motor cortex and lumbar spinal cord) and in human ALS (eg mutant SOD1 and C9orf72) iPS astroglia.

Discussion and conclusion: How these astroglial-unique pathways play a role in cortical and spinal cord neuronal injury in ALS is being investigated. This is one of the first studies of astroglial biology to exploit a defined subtype that is directly involved with human and rodent ALS motor neuron disease. We hope that elucidation of this astroglia subtype will provide us with tools to use cell-specific approaches to target ALS motor neuron disease.

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IVV-12 Role of connexin 43 in disease progression and motor neuron toxicity in a rodent model and human iPS astrocytes in amyotrophic lateral sclerosis

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Keywords: connexin 43, human iPS astrocytes, $SOD1^{G93A}$ mouse model

Background: Astrocytes are altered in ALS and in turn affect motor neuron (MN) survival and disease progression. Astrocytes are interconnected through connexins (Cx) to form hemichannels and gap junctions. Cx43 is a major astrocyte connexin conducting crucial homeostatic functions. Our recent work demonstrates a significant increase in Cx43 expression in rodent and human ALS tissues and Cx43 mediates toxicity to MNs and Cx43 blockers result in neuroprotection *in vitro.*

Objective: Our current work is understanding the role of Cx43 in disease progression using an *in vivo* mouse model and also in sporadic and familial human iPS astrocytes.

Methods: To understand the role of Cx43 *in vivo*, we used the SOD1^{G93A} mouse model and generated a transgenic mouse with conditional loss of Cx43 in astrocytes using a human GFAP-Cre driver mouse referred to as SOD1^{G93A}::Cx43KO mice. We conducted behavioral, survival and histological studies on the SOD1^{G93A}::Cx43KO mice. In addition, we are examining human iPS astrocytes to further understand the role of Cx43 in sporadic ALS patients along with familial patients.

Results: We examined that specifically deleting Cx43 in astrocytes resulted in a modest yet significant prolongation in survival of SOD1^{G93A} mice. The motor function of SOD1^{G93A}::Cx43KO mice and littermate control SOD1^{G93A} was tested using grip strength analysis and SOD1^{G93A}::Cx43KO mice displayed significantly conserved forelimb grip strength while hind limb function was comparable between the two groups. We further examined preservation of MNs in these mice at different stages of disease progression. We observed no change in the number of MNs in the lumbar spinal cord; however, a significant preservation of MNs was observed in the cervical spinal cord of SOD1^{G93A}::Cx43KO mice compared to SOD1^{G93A} mice at the end stage.

Discussion and conclusions: While *in vitro* studies show blocking Cx43 results in MN protection, *in vivo* studies implicate that Cx43 is potentially involved in disease progression at least in the SOD1^{G93A} mice. The lack of change in preservation of lumbar MNs and hind limb function between the SOD1^{G93A}::Cx43KO and SOD1^{G93A} mice, indicates that Cx43 does not contribute to disease onset. However, conserved forelimb motor function and MNs in the cervical cord of SOD1^{G93A}::Cx43KO mice indicate a slower rate of disease progression compared to SOD1^{G93A} mice. Current studies are focused on modeling ALS using human iPS astrocytes to understand the role of astrocyte Cx43 in sporadic forms of ALS and dissect its role in disease progression. We are also investigating potential mechanisms through which Cx43 mediates toxicity on MNs. These studies have widespread implications in not just ALS but also other neurodegenerative diseases involving astrocyte mediated effects.

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IVV-13 Beneficial effect of oxaloacetate for the neuromuscular function of SOD1^{G93A} mice

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Keywords: mitochondria, neuromuscular strength, SOD1G93A mice

Introduction and aims: The etiology of ALS remains unknown despite the identification of mutated genes in familial and/or sporadic ALS. It is thought that aggregates of disease-causing mutant proteins and RNAs disrupt the normal cellular functions that leads to dying back neuropathy and motor neuron degeneration. Mitochondrial dysfunction is thought to play a major role in the pathology of ALS (1–3). Therefore, restoration of mitochondrial function is likely to cause beneficial effects for the survival of ALS model mice (4).

The objective of this project is to evaluate the effects of oxaloacetate for the neuromuscular function and lifespan of the superoxide dismutase 1 (SOD1) ^{G93A} transgenic mice. Oxaloacetate has beneficial effects on mitochondria biogenesis. Systemic administration of oxaloacetate to wild-type mice increased brain expression level of peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α), which is a transcriptional co-activator and a master-coordinator of mitochondrial biogenesis (5). Furthermore, oxaloacetate altered levels, distributions or post-translational modifications of proteins and mRNAs in ways that promote mitochondrial biogenesis (PGC1 related co-activator, NRF1, etc.) (5). Oxaloacetate can access the central nervous system by systemic administration in mice (5,6), is neuroprotective in neurodegenerative model mice induced by kainic acid administration (6,7), and prolongs survival of wild-type *C. elegans* by mimicking caloric restriction (8). However, oxaloacetate has not been tested alone in transgenic rodent models of ALS.

Methods: The oxaloacetate effect was compared in three groups: oxaloacetate treatment from the pre-symptomatic stage, oxaloacetate treatment from the symptomatic stage, and the control group without oxaloacetate. Oxaloacetate was administered systemically to SOD1^{G93A} mice by daily intraperitoneal injection. To evaluate the neuromuscular function, behavior tests were performed prior to oxaloacetate treatment and every 10 days during the treatment until the end stage of the lifespan.

Results and discussion: The pre-symptomatic stage treatment significantly improved the neuromuscular strength. The symptomatic stage treatment significantly delayed the limb paralysis and showed a trend of lifespan expansion. Oxaloacetate treatment from the pre-symptomatic stage group was also analyzed for mitochondrial activity levels using an Oroboros high resolution respirometer and for expression levels of mitochondria biogenesis related proteins. These data suggest that oxaloacetate treatment has a beneficial effect to maintain the neuromuscular function in ALS model mice. This work was supported by grants from KCALSI (HN) and NIH R01NS078214 (HN).

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IVV-14 Increasing urate levels may delay disease onset in the SOD1 G93A mouse model of amyotrophic lateral sclerosis

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Keywords: SOD1, urate, oxidative stress

Background: An increase in oxidative stress has been implicated as an important pathogenic mechanism in the onset and progression of ALS. Thus, an improved understanding of oxidative stress-induced motor neuron death may lead to mechanism-based therapies. One of our major endogenous defenses against oxidative stress is urate, an antioxidant and byproduct of purine metabolism. Recent findings have highlighted the neuroprotective potential of urate in neurodegenerative diseases such as Parkinson's disease (PD), Huntington's disease (HD), Alzheimer's disease (AD) and ALS. Specifically, urate was shown to be a strong molecular predictor for both reduced risk (PD, AD) and favorable progression (PD, ALS, HD). Furthermore, urate elevation was found to be neuroprotective in pre-clinical PD models. However, little is known about the neuroprotective effects of urate in ALS.

Objectives: In this study, we sought to assess the neuroprotective potential of urate and determine whether increases in urate levels could delay onset, prolong survival, or attenuate motor deficits of ALS in the G93A SOD1 mouse model.

Methods: To increase CNS urate levels, mice with a mutation in the gene (*UOx*) encoding urate oxidase, an enzyme responsible for urate metabolism, were crossed with transgenic (Tg) SOD1 G93A mice to generate Tg *SOD1/UOx*$^{-/-}$ mice and littermate controls. We assessed body weight, neurological score, motor function, disease onset and progression. In addition, in order to validate the potentially beneficial effects of urate in the context of human patient motor neurons we assessed its ability to protect mutant SOD1 and isogenic control motor neurons *in vitro*.

Results: Our results demonstrate that elevated urate levels are associated with a 20-day delay in the onset of hind limb paresis in the Tg SOD1/UOx$^{-/-}$ mutant mice compared to Tg SOD1/UOx$^{+/+}$ or wild-type littermates. In addition, pretreatment of patient-derived motor neurons with urate significantly protected against glutamate and oxidative stress-induced toxicity.

Discussion and conclusions: These findings demonstrate that increasing urate levels has an impact on the progression of symptoms in the G93A SOD1 mouse model, and attenuates motor neuron loss caused by oxidative stress and excitotoxicity. Ongoing studies are assessing the effects of increased urate on motor neuron counts and neuromuscular junction integrity in order to further elucidate the therapeutic potential of urate in ALS.

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IVV-15 Circadian rhythm dysfunction accelerates disease progression and increases intestinal cyanobacteria in an amyotrophic lateral sclerosis model

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Keywords: circadian rhythm dysfunction, cyanobacteria, intestine

Background: Sleep disorders such as nocturnal hypoventilation, sleep-disordered breathing and circadian disturbances (CD) appear in ALS patients. The disturbed rhythm that derives from an abnormal light/dark cycle can destroy the peripheral rhythmicity, and alters intestinal bacterial compositions. In particular, the gut cyanobacteria are considered as a cause for regional clustering of ALS patients (1–4).

Objectives: We firstly explore the negative consequences and a possible mechanism regarding gut microflora of exposure to CD in ALS mice in this study.

Methods: The SOD1^{G93A} and wild-type mice were respectively divided into four groups: the ALS and WT groups were kept on a regular 12 h/12 h light/dark cycle, while the ALS + CD and WT + CD groups underwent a 20 h/4 h light/dark cycle from day 42. Three endpoints were set, respectively, at 60, 90 and 120 days after birth. Behavior assessments like weight and motor functional detections were acquired every three days until the endpoints. Motor neuron loss and astrocyte activation in the anterior horn of the lumbar spinal cord were detected by immunofluorescence. Fresh fecal samples were collected and 16 S ribosomal RNA (r RNA) gene sequencing was performed to characterize the distal gut microflora.

Results: Circadian rhythm disorder accelerated disease progressions of ALS mice, seen through faster weight loss, earlier onset of paralysis, shorter survival time, exacerbated motor neuron apoptosis and aggravated astrocyte activation in the spinal cord along with disease progression. Also, compositions of intestinal microbiota varied at different stages of disease progression, at phylum level, and the relative abundance of cyanobacteria increased after early disease onset (day 90, $p<0.05$) in SOD1G93A mice. Moreover, further-promoted abundance of gut

cyanobacteria were shown in ALS + CD mice, especially at day 60 and day 90 (<0.05) compared with ALS mice.

Discussion and conclusions: Our results provide an initial record of varied intestinal microflora, especially the relative abundance of gut cyanobacteria in SOD1G93A mice. Also, circadian rhythm disorder promoted disease onset and progression of ALS, and one of the valid mechanisms could be the further-doubled increase of gut cyanobacteria in SOD1G93A mice. It may reveal that CD and cyanobacteria are co-connected risks and represent potential therapeutic targets for ALS.

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IVV-16 Corticospinal motor neuron degeneration precedes spinal motor neuron degeneration and involves a new set of molecular players

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Keywords: corticospinal motor neurons, spatiotemporal kinetics, transcriptomics

Background: Amyotrophic lateral sclerosis (ALS) is clinically defined by the combined degeneration of 2 neuronal populations: the corticospinal motor neurons (CSMN) and the spinal and bulbar motor neurons (SMN). Despite this precise description, little is known about the spatiotemporal regulation of CSMN degeneration, and the molecular mechanisms behind it.

Objectives: The work aims to better characterize the kinetics of CSMN versus SMN degeneration, and to decipher the molecular mechanisms that selectively trigger CSMN dysfunction and death during the course of ALS.

Methods: To characterize the spatiotemporal regulation of CSMN degeneration, we retrogradely labelled the CSMN either from the cervical, or the lumbar part of the spinal cord of wild-type and $Sod1^{G86R}$ mice (1); and quantified the corresponding population and subpopulation at pre-symptomatic and symptomatic ages. The molecular mechanisms underlying CSMN degeneration have been investigated, by RNAseq on pure populations of CSMN isolated from the cerebral cortex of adult wild-type and $Sod1^{G86R}$ mice, at different stages of the disease, and compared to callosal projection neurons (CPN) from the same animals.

Results: Patients who carry a mutation of the *SOD1* gene present with an initial paralysis that affects the legs. Similarly, $Sod1^{G86R}$ mice present with an initial alteration of the hind limbs. We show that $Sod1^{G86R}$ mice recapitulate CSMN progressive degeneration, and that CSMN with a lumbar projection, ie that project to the portion of the spinal cord where SMNs that innervate the hind limbs are located, are affected earlier and to a greater extent than the whole CSMN population, in accordance with a somatotopic relationship between cortical and spinal insults. Importantly, we show that CSMN loss starts pre-symptomatically and precedes SMN degeneration and neuromuscular junction denervation.

We developed a method to purify in parallel the CSMN and the CPN from the cerebral cortex of the same, individual adult mouse brains, under physiological or neurodegenerative conditions, in order to conduct comparative transcriptomic analyses between disease-sensitive and disease-resistant cortical neuron populations. Our RNAseq results reveal pre-symptomatic alterations of the CSMN transcriptome, and unravel the involvement of genes that have never been described in the context of ALS.

Discussion and conclusions: The work is intended to provide, for the first time, a comprehensive molecular picture of CSMN as they degenerate during the course of ALS, and may inform the development of alternative therapeutic strategies for ALS and related CSMN-specific neurodegenerative diseases.

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IVV-17 Early dysfunction of premotor glycinergic interneurons in a zebrafish model of amyotrophic lateral sclerosis

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Keywords: zebrafish, SOD1, interneurons

Background: There is now clear evidence for a prolonged preclinical phase in ALS that involves progressive pathology in motor neuron and glial populations. However, the relevance of interneurons to preclinical pathology remains poorly understood. To address this problem, we are using a SOD1 G93R zebrafish model of ALS that harbours a genetically encoded stress reporter (HSP70:DsRed). We have previously found that stress responses are selectively up-regulated in interneuronal populations of presymptomatic fish (1). Moreover, we find the majority of stressed interneurons are glycinergic, suggesting that stress in these cells is a presage for disease. However, the relationship between stress and neuronal dysfunction has not been investigated.

Method: Here, we use *in vivo* patch clamping to probe for excitability and membrane defects in stressed glycinergic interneurons of presymptomatic SOD1 mutant zebrafish.

Results and discussion: Compared to glycinergic interneurons of control fish, we find that SOD1-mediated stress is associated with increased input resistance, decreased capacitance and uncontrolled generation of prolonged, rapidly inactivating action potentials. These defects occur in conjunction with a decrease in glycinergic drive to spinal motor neurons. Analysis of voltage gated currents revealed a depression of potassium conductances in stressed interneurons, which could account for the firing defects we observe. In contrast, patch clamping of spinal motor neurons revealed no evidence of dysfunction at this stage of the disease.

Conclusion: Our findings point to early deficits in glycinergic interneuron function during presymptomatic ALS. We posit that, over time, these changes may exacerbate motor neuron stress and accelerate progression to symptomatic stages of the disease.

Acknowledgements: This work was supported by a Motor Neurone Disease Association project grant.

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IVV-18 Environmental and genetic contributions in an ALS model: failed recovery and enhanced ventral horn inflammation after peripheral nerve injury

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Keywords: injury, animal model, disease progression

Background: ALS is poorly understood and no effective therapeutics exist to stop its insidious progression. For some it starts in a hand or a foot, and in others with trouble swallowing. Once it starts the disease progresses up and down the spinal cord until respiratory failure leads to death or mechanical ventilation. The clinical symptoms progress as the disease spreads slowly over years. Clinicopathologic human studies have shown a clear relationship between disease onset and lower motor neuron loss, with the most severe lower motor neuron loss at the site of disease onset. One long entertained observation is that ALS may be precipitated by nerve or brain injury. Many patients with ALS are athletes (Lou Gehrig) and may have suffered minor nerve injuries in the limb where ALS first presents. However, it is unclear whether and how nerve injury plays a role in ALS development. A growing number of animal models for ALS are based on human genetic forms of the disorder, but a large number of therapeutic interventions that seemed to work in these models have all failed in human clinical trials. Our laboratory and others have previously shown an increase in spinal cord microglial activation in post-mortem ALS patient tissue, as well as at an early stage in animal models of ALS, suggesting a microglial activation contribution in the early stage of neurodegeneration.

Aim: Our current study seeks to link an environmental factor (nerve injury) with a genetic factor (SOD1 mutation) to induce symptom onset and disease progression.

Method: We performed sciatic nerve crush injuries in SOD1 G93A rats and age, sex and background matched wild-type controls at an early stage of their disease, prior to any known symptoms. Functional recovery using the EPT test was tracked weekly following surgery. Spinal cord tissue was collected at different stages of disease for tissue staining and quantitative analysis of microglia and motor neurons.

Results and discussion: Significantly enhanced and sustained microglial activation was seen in the ventral horns of SOD1 rats 1–2 weeks after injury that spread to nearby, uninjured motor neuron pools. Microglial activation subsided by seven weeks, however, while the wild-type animals fully recovered within three weeks, the SOD1 animals never recovered functionally. Long-term effects of this early injury on survival and the underlying mechanism by which enhanced inflammatory mechanisms lead to failed recovery from injury are underway. These studies take a unique approach to understand the effects of early

environmental contributions (nerve injury) in a genetic model of ALS. The animal model developed could be an important new model for drug development that focuses on disease onset and progression rather than traditional models of survival that may better translate to the human condition.

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IVV-19 Identifying molecular drivers of ALS in transgenic TDP-43 mice

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Keywords: TDP-43, mouse model, proteomics

Background: Most cases of ALS are characterized pathologically by accumulation of phosphorylated cytoplasmic TAR DNA binding protein of 43 kDa (TDP-43) in affected neurons. However, the early drivers of pathogenesis have remained largely unclear, in part due to a lack of valid mouse models that recapitulate the key features of ALS. However, a new transgenic mouse model inducibly expressing cytoplasmically-targeted TDP-43 under the control of the neurofilament heavy chain promoter, known as the rNLS mouse, develops both progressive neurodegeneration and motor phenotype with accumulation of pathological TDP-43 in the brain and spinal cord similar to ALS (1).

Objectives: We aimed to determine the key proteins involved in onset and progression of disease in the rNLS TDP-43 mouse model.

Methods: We used quantitative sequential windowed data-independent acquisition of the total high-resolution mass spectra (SWATH-MS), to screen proteins extracted from brains and spinal cords of rNLS TDP-43 mice at early, mid and late stages of disease, with age- and litter-matched non-transgenic controls ($n > 3$/group). Ingenuity Pathway Analysis was used to identify altered biochemical networks. Identified individual targets were validated by immunoblotting, qPCR, immunofluorescence and confocal microscopy.

Results: SWATH-MS quantitatively detected multiple peptides from up to ~2500 individual proteins per sample. We identified >240 proteins as either significantly increased or significantly decreased by >1.5-fold (q-value <0.1) in at least one region/time-point in rNLS TDP-43 mice compared to controls, with distinct but overlapping signatures through disease progression. Multiple pathways, including those related to synaptic function, intracellular transport and mitochondrial function, were altered in rNLS TDP-43 mice, suggesting diverse biochemical changes even at very early stages of disease, progressing to later activation of neuronal apoptotic signaling.

Discussion and conclusions: These studies reveal a signature of the most upstream pathological events caused by accumulation of cytoplasmic TDP-43, and time-course analysis allows dissection of early and late changes. These findings therefore provide insight into both instigators of neurodegeneration and the biochemical drivers of disease progression. Mechanistic studies of targets in neuronal cell culture and by immunoblotting and immunofluorescence, in human brain and spinal cord samples, will further clarify individual proteins that directly and indirectly regulate TDP-43 accumulation and ALS-related pathology. These studies thereby offer possibilities for the identification of new therapeutic avenues for this devastating disease.

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IVV-20 Using in-cell NMR to study the protein folding and structural dynamics of TAR DNA binding protein-43

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Keywords: TDP-43, NMR, protein folding

The presence of insoluble proteinaceous aggregates is a key pathological hallmark found in the motor neurons of both familial and sporadic amyotrophic lateral sclerosis (ALS) patients. Trans-activation response DNA binding protein-43 (TDP-43) has been identified as the major pathological protein of ALS, but it is currently not known exactly how this protein is involved in the pathogenesis of the disease. TDP-43 is a 440-residue, multi-domain protein with a disordered C-terminal region ($CTD_{274-414}$)

that propagates the aggregation of TDP-43 and hosts almost all disease-associated mutations. There is currently no 3D structural information of the TDP-43 protein or detailed understanding of how the CTD region mediates aggregation of the protein. To initiate structural studies of TDP-43, we have developed a recombinant expression and purification strategy to produce the $CTD_{274-414}$, and we apply NMR spectroscopy which is unique in its capacity to study dynamic systems at high resolution. In particular, we have been developing an in-cell NMR strategy which can observe proteins *in situ* within living cells. This approach enables us to study the structural and dynamic properties of TDP-43 as it exists within a native cellular environment.

Our study provides a basis to derive a high-resolution structural understanding of TDP-43 and the process by which it transitions from a functionally native protein to cytotoxic aggregates, as observed in the early stages of ALS.

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IVV-21 Identifying physiologically relevant targets of TDP-43 translational inhibition

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Keywords: TDP-43, translation, inhibition

Background: The mRNA binding protein TDP-43 forms cytoplasmic inclusions as part of the pathogenesis of amyotrophic lateral sclerosis (ALS), a neurodegenerative disorder affecting motor function and survival. A potential causative mechanism for the ALS phenotype is inhibition of key metabolic enzymes via translational repression (1).

Objective: The objective of the project was to identify physiologically relevant targets of TDP-43 translational inhibition.

Methods: We overexpressed TDP-43, either wild-type or a mutant variant, in *Drosophila* motor neurons (2), then performed immunoprecipitation experiments to detect mRNAs enriched in TDP-43 complexes. Tagged Ribosome Affinity Purification, transcriptomics, and metabolomics were also employed to understand the molecular and metabolic changes caused by TDP-43 overexpression in *Drosophila* motor neurons.

Results: Several mRNA candidates linked to synaptic function and metabolism were identified as potential primary targets due to their high association with TDP-43 (log-fold change >2 and padj <0.05). DAVID (Database for Annotation, Visualization and Integrated Discovery) analyses identified several pathways including the tricarboxylic acid (TCA) cycle, oxidative phosphorylation, and amino acid catabolism. We are currently validating the top candidate targets using quantitative PCR, expression analyses and genetic interactions.

Conclusions: These findings support changes in metabolic gene expression that provide an explanation for deficits in cellular energetics in ALS. Current experiments are aimed at validating these findings in other model systems and examining the physiological relevance of these targets in flies and human cell based models and tissues.

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IVV-22 PI3Kα/mTOR pathway rescues TDP-43 toxicity in the spinal motor neuron in zebrafish

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Keywords: TDP-43, PI3Kα, mTOR

Background: TAR DNA-binding protein 43 (TDP-43, *TARDBP*) is a major component of the cytoplasmic inclusions that are a pathological hallmark of amyotrophic lateral sclerosis (ALS). While the spinal motor neuron is a major cell type that is targeted for degeneration in ALS, how TDP-43 toxicity leads to dysfunction and degeneration of the spinal motor neuron has not been fully understood.

Objectives: To characterize TDP-43 toxicity in the spinal motor neuron *in vivo*, and to explore a method of alleviating the toxicity.

Methods: We established a transgenic zebrafish line labeling a genetically defined spinal motor neuron subtype

CaP with Gal4 transcription factor, allowing for visualizing and genetically manipulating CaP *in vivo*. By using this line, we established two zebrafish ALS models. First, we knocked out two zebrafish TDP-43 genes (*tardbp* and *tardbpl*) by the CRISPR-Cas9 method. Secondly, we overexpressed TDP-43/*tardbp* specifically in CaP by Gal4/UAS-mediated gene expression. By taking advantage of the translucency of zebrafish larvae, we monitored the entire CaP over time under both conditions, with genetic and pharmacological modifications.

Results: We found that both systemic knockout and targeted overexpression of TDP-43 resulted in an axonal outgrowth defect and reduction in motor unit size of CaPs. By exploring biological pathways that could rescue the toxicity associated with these TDP-43 manipulations, we found that the targeted activation of PI3Kα effectively restored the axonal outgrowth of CaPs overexpressing TDP-43. The defects in neuromuscular synapse formation and neuronal excitability were also rescued, albeit partially, by the PI3Kα activation. We further found that the bath application of rapamycin, an inhibitor of the mTOR protein kinase, blocked the PI3Kα-mediated restoration of the axonal outgrowth, suggesting that mTOR is a downstream effector of the rescue effect. Intriguingly, while rapamycin treatment inhibited the axonal outgrowth of CaPs in the wild-type fish, it did not exacerbate the axonal outgrowth defect caused by TDP-43 overexpression, implying that TDP-43 causes the axonal outgrowth defect through inhibiting the mTOR pathway.

Discussion and conclusions: These observations suggest that an elevated TDP-43 level hampers axonal outgrowth through attenuating the PI3Kα/mTOR pathway that promotes axonal outgrowth in the spinal motor neuron. Therefore, activation of the PI3Kα/mTOR pathway might serve as a potential strategy to maintain or restore motor unit size in ALS.

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IVV-23 ALSci associate pathological phosphorylation of Thr^{175} tau induces a tau proteinopathy *in vivo*

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Keywords: neuropsychology, rat model, tau

Background: We have previously shown that amyotrophic lateral sclerosis with cognitive impairment (ALSci) is associated with pathological tau phosphorylation at Thr^{175} (1). *In vitro* expression of pseudo phosphorylated human tau (Thr^{175}Asp tau) leads to tau fibril formation and cell death mediated through Thr^{175}Asp tau induction of GSK3β which in turn induces Thr^{231} phosphorylation – the consequence of which is fibril formation (2,3). We have also demonstrated that this pathological processing of tau protein, driven by $pThr^{175}$, is a feature of multiple tauopathies (4).

Objectives: To determine whether $pThr^{175}$ human tau, when introduced *in vivo* using somatic gene transfer with recombinant adeno-associated virus (rAAV9) induces a tauopathy.

Methods: Adult female Sprague-Dawley rats were injected bilaterally in the hippocampus with GFP-tagged tau construct-bearing rAAV9. Four groups of 10 rats were injected with either 1) GFP, 2) GFP-WT tau, 3) GFP-Thr^{175}Ala tau (cannot be phosphorylated), or 4) GFP-Thr^{175}Asp tau. Behaviour was assessed at 1, 3, 6, 9, and 12 months post injection, by Morris water maze, open field, and startle box testing. Magnetic resonance imaging (MRI) was conducted on a 9.4 T MRI scanner and diffusion tensor imaging was assessed at 1, 3, 6, 9, and 12 months post injection. At six and 12 months post injection pathology was investigated by immunohistochemistry (IHC) using antibodies against GFP and $pThr^{231}$ tau.

Results: We observed robust uptake of the rAAV9 construct throughout the hippocampus of all inoculums. GFP-tagged human tau expression was most prominent in the CA2 region at both six and 12 months post injection in all groups. In those rats expressing GFP-Thr^{175}Asp, we detected a range of pathological tau inclusions using antibodies against GFP and $pThr^{231}$ tau. This included fibrillar inclusions, plaques, neurofibrillary tangles, torsional, and beaded axonal processes. This pathological tauopathy was observed to a greater degree at month 12 than month six, and only in the $pThr^{175}$-Asp inoculated group with the exception of rarely observed beaded axonal pathology in rats regardless of inoculum at month 12. No abnormalities were observed on either neuroimaging or behavioral testing.

Discussion and conclusions: We have demonstrated that pseudophosphorylated tau ($pThr^{175}$Asp) induces a neuropathological tauopathy *in vivo*, replicating the cell-specific neuropathological observations of ALSci. The absence of either neuroimaging or behavioral pathology reflects the highly restricted nature of the pathology, a feature that would be predicted to be overcome in long-term survival studies. These studies also suggest that the pharmacological inhibition of GSK3b activity, previously shown to inhibit pThr175-Asp tau fibril induction and toxicity *in vitro* should be considered in the therapy of ALSci.

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IVV-24 CSF and serum neurofilament light chain levels as a biomarker for diagnosis and disease progression in a canine disease model of ALS

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Keywords: biomarker, neurofilament, dog

Background: Canine degenerative myelopathy (DM) is a late-onset, progressive neurodegenerative disease affecting many pure and mixed-breed dogs. Clinical signs are the result of multisystem neurodegeneration involving the central and peripheral nervous systems. DM initially manifests as spastic upper motor neuron paraparesis and general proprioceptive ataxia (stage one). Progressive neurodegeneration results in non-ambulatory paraparesis/paraplegia (stage two) and thoracic limb paresis (stage three). End-stage disease culminates in flaccid tetraplegia, widespread muscle atrophy and bulbar dysfunction (stage four). Superoxide dismutase 1 gene (*SOD1*) mutations (*SOD1:c.118A, SOD1:c.52T*) are risk factors for DM, with most cases resulting from autosomal recessive inheritance. The disease is considered to be similar in cause, progression, and prognosis to some forms of human amyotrophic lateral sclerosis (ALS). Diagnosis is based on exclusion of other mimicking diseases. Thus, a definitive diagnosis of DM can only be made post mortem by histopathologic examination of the spinal cord. Neurofilament light chain (NFL), an abundant structural protein of myelinated motor axons, is a promising fluid biomarker of animal and human motor neuron diseases, including ALS. Blood and CSF NFL levels have diagnostic value in ALS, and may correlate with disease progression and thereby serve as a biomarker of treatment effect.

Objectives: To assess serum and CSF NFL levels as a potential biomarker for diagnosis and disease progression in DM dogs.

Methods: Archived CSF and serum samples from young and aged (>9 years) normal control dogs, DM-affected dogs (homozygous for the SOD1c.118A allele) of stage one through four, and asymptomatic dogs homozygous for the SOD1c.118A allele were analyzed. NFL concentrations were determined with a commercially available digital ELISA for human NFL, which is highly conserved with canine NFL. Prior to sample testing, the ELISA assay was assessed for linearity, sensitivity, stability, spike recovery and inter-assay variability.

Results: The ELISA assay was optimized to meet predefined acceptance criteria across assessed parameters for each matrix.

CSF NFL levels were elevated substantially in DM-affected dogs of stages 1–4 compared to controls. Similar trends were observed in serum. Furthermore, CSF NFL levels increased with disease severity (stage). Serum NFL levels showed less pronounced elevation with disease progression. In control dogs, CSF and serum NFL levels were linearly correlated.

Discussion and conclusions: Our results suggest that NFL concentration in CSF and serum may serve as a diagnostic marker of DM. CSF NFL concentrations correlate with disease progression and may represent a biomarker for disease progression.

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IVV-25 Embryonic exposure to the environmental neurotoxin BMAA negatively impacts early neuronal development and progression of neurodegeneration in the Sod1-G93R zebrafish model of amyotrophic lateral sclerosis

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Keywords: zebrafish, BMAA, gene-environment

Background: The scientific consensus is that gene-environment interactions are key for the development and progression of ALS, but how either toxicants or genes lead to a disease mechanism is currently unknown. A suite of environmental neurotoxicants has been associated with ALS, with evidence indicating that early developmental exposures to neurotoxins can have consequences for neurotoxicity later in life. Early defects in neural circuitry have also been found to be associated with late-onset neurological disorders, including both cognitive and degenerative diseases. By determining cellular pathways involved in modifying neurological defects we hope to gain a better understanding of the root causes of this disorder.

Objectives: Our research aims to study the intersection of genetics and environmental neurotoxins on both developmental motor neuron defects and on adult-onset disease in a zebrafish model of ALS.

Methods: We have determined the impact of embryonic exposure to environmentally relevant doses (0–25 μg/L) of the ALS-associated cyanobacterial neurotoxin Beta-methylamino-L-alanine on neurodevelopmental defects in mutant SOD1-ALS zebrafish, and the consequences for adult motor function.

Results: 1) Tg-SOD1-G93R zebrafish exhibit significantly shorter 30hpf motor neurons at medium doses (10 μg/L BMAA) but Tg-SOD1-WT overexpressing embryos are not impacted at all. 2) 5-month-old Tg-SOD1-G93R fish (embryonically exposed to BMAA) show decreased ability to swim against water current, with increasing embryonic BMAA dose having a negative impact on swimming ability. In contrast, Tg-SOD1-WT fish exhibit an increased swimming ability with increasing BMAA dose. 3) 5-month Tg-SOD1-G93R fish also show increasing fatigue when repeatedly challenged in the water current, while Tg-SOD1-WT fish do not exhibit any change in swimming over repeated challenges.

Discussion: Our results indicate that genetic and environmental insults combine to facilitate neurological dysfunction in ALS, and that overexpression of wt-SOD1 may have protective effects against neurotoxin damage. The defects seen in early neurodevelopment are mirrored at five months of age in the ability of fish to swim against a current and to fatigue with repeated swimming challenges. Establishing these links between exposure and adult motor neuron disease increases the power of the zebrafish model for toxicological and drug screens.

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IVV-26 Assessing the role of sense and antisense foci in *Drosophila* models of C9orf72 ALS/FTD

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Keywords: C9orf72, *Drosophila, foci*

Background: A GGGGCC hexanucleotide repeat expansion in the C9orf72 gene is the most common genetic cause of both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The hexanucleotide repeat region is transcribed in both sense and antisense direction, producing hexanucleotide RNA molecules, as well as being translated into five repetitive dipeptide repeat proteins via repeat-associated non-ATG initiated translation (RANT). Neurotoxicity has been proposed to occur via three non-mutually exclusive mechanisms: loss of function of the C9orf72 gene, or toxicity caused by either sense/antisense RNA or dipeptide repeat protein(s) (1). We have previously demonstrated that expression of GGGGCC repeat mRNA is extremely toxic to *Drosophila*. Crucially, this toxicity is prevented when stop codons are inserted along the repeat region length ('RNA only' models), indicating that dipeptide proteins cause toxicity in our existing *Drosophila* models (2).

Objectives: The ability of RNA only constructs to suppress RANT makes them ideal to further study the potential role of RNA toxicity in disease. We hypothesized that the genomic context, or repeat length of hexanucleotide RNA may alter its properties, and thus its propensity to be toxic.

Methods and results: We have created novel *Drosophila* models capable of expressing sense or antisense ~100 RNA only repeats as either part of a processed mRNA containing a polyA tail, or as part of a constitutively spliced intronic region of an eGFP mRNA transcript. We have found that while repeat polyA mRNA is largely cytoplasmic in localization, intronic RNA is predisposed to form RNA foci. However, despite mimicking the pathology seen in human patients, neither sense or antisense cytoplasmic RNA or RNA foci cause an effect on climbing ability or reduce lifespan when expressed in adult *Drosophila* neurons. Patients with the disease typically carry hundreds to thousands of repeats. Thus, as an additional attempt to model disease more accurately, we have created *Drosophila* expressing >1000 sense RNA only repeats. These flies form very large numbers of RNA foci and sequester *Drosophila* RNA binding proteins. However, we fail to observe an effect on climbing ability, or a reduction in lifespan when expressed in adult *Drosophila* neurons.

Discussion and conclusions: These results suggest that in human disease, toxicity may be primarily driven by the formation of dipeptide repeat proteins. However, we do not rule out a less potently toxic role for sense and antisense repeat RNA and are utilizing our novel fly lines to investigate this possibility further.

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IVV-27 Activation of BMP signaling in non-motor neurons rescues motor dysfunction in a Drosophila model of amyotrophic lateral sclerosis

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Background: Defects in several cellular mechanisms likely contribute to amyotrophic lateral sclerosis progression, but deciphering which changes occur first and which are secondary consequences has been difficult since most cellular processes are already affected when a patient is diagnosed. To study altered cellular processes early in disease progression, we have taken advantage of a *Drosophila Superoxide dismutase 1* (*dSod1*) knockin model that contains a mutation synonymous to the human $SOD1^{G85R}$ (1).

Objectives: Our first objective is to identify cellular processes that are disrupted early in disease progression. Our second objective is to prevent or compensate for disrupted cellular processes and determine how these changes affect disease onset and progression.

Methods: Phenotypes were evaluated using behavioral assays, electrophysiology, and immunofluorescence.

Results: Late stage $dSod1^{G85R}$ animals have locomotor dysfunction, neuromuscular junction (NMJ) degeneration, decreased neurotransmission across the NMJ, and reduced lifespan. Interestingly, earlier we observed locomotor dysfunction without motor neuron degeneration or major defects in neurotransmission to muscle. Instead, we observe defects in feedback from the peripheral nervous system to the central nervous system locomotor pattern generator. The observation of electrophysiological defects in non-motor neurons that precede major motor neuron degeneration begs the question as to whether these non-motor neuron defects influence motor neuron degeneration and disease progression.

To compensate for the motor neuron degeneration observed in $dSod1^{G85R}$ animals, we activated a signaling pathway known to stimulate neuronal growth, the Bone Morphogenetic Protein (BMP) signaling pathway. Cell-autonomous activation of BMP signaling in either the glutamatergic motor neurons or upstream cholinergic neurons improved the motor function of $dSod1^{G85R}$ animals and the activation of BMP signaling in cholinergic neurons was also able to extend survival.

Conclusions: The observation of dysfunction in non-motor neurons is consistent with patient studies showing sensory neuron (2) and interneuron disruption (3). Future studies will: 1) determine how circuitry changes influence the onset and progression of ALS-like phenotypes; and 2) identify which cellular processes, regulated by BMP signaling, alleviate motor dysfunction.

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IVV-28 Stress leads to neurodegeneration in single-copy models of amyotrophic lateral sclerosis in *C. elegans*

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Keywords: SOD1, C. elegans, FUS

Background: A common cause of familial ALS (FALS) is the mutation of Cu/Zn superoxide dismutase 1 (SOD1). SOD1 containing patient mutations (FALS SOD1) leads to intra-neuronal aggregates and neurodegeneration. Overexpression of FALS SOD1 in *C. elegans* leads to synaptic dysfunction and dramatic SOD1 aggregation (1), consistent with a gain of toxic function in FALS. However, overexpression models may obscure the potential impact of diminished FALS SOD1 activity in early stages of disease. Thus, it remains unclear how altered FALS SOD1 activity affects neuronal function prior to neurodegeneration.

Aim and methods: Here, we examine the impact of FALS SOD1 mutations in single-copy, FALS SOD1 knock-in models in *C. elegans*. Using MosSCI and CRISPR mediated homologous recombination, we edited the endogenous *sod-1* gene to incorporate the ALS mutations corresponding to A4V, H71Y, L84V, G85R and G93A. The wild-type SOD1 enzyme protects the cells from oxidative damage. To test FALS *sod-1* function *in vivo*, we subjected animals to oxidative stress.

Results and discussion: FALS *sod-1* alleles resulted in neurodegeneration, to varying degrees, in cholinergic and glutamatergic neurons after exposure to oxidative stress. Furthermore, oxidative stress leads to degeneration of glutamatergic neurons in animals lacking *sod-1* as well as in a subset of FALS *sod-1* animals, suggesting that decreased *sod-1* function may contribute to neuronal degeneration in FALS. Intriguingly, oxidative stress leads to degeneration of cholinergic motor neurons only in FALS *sod-1* animals but did not affect neuronal survival in

animals lacking *sod-1*, suggesting a gain of the toxic role in FALS *sod-1*. We found that all FALS *sod-1* alleles increased aggregation of human SOD1 in motor neurons. Combined, our results suggest that exposure to exogenous stressors may unmask or aggravate the defects associated with FALS SOD1. Also, our findings show that both loss and gain of toxic *sod*-1 function may be involved in disease pathogenesis, albeit to differing extents for different FALS *sod-1* alleles.

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IVV-29 Identification of suppressors of stress-induced neurodegeneration in a knockin SOD1 model

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Keywords: SOD1, genetic modifiers

Background: Of the approximately 10% of ALS cases that have a familial inheritance pattern (FALS), 20% of FALS cases result from mutations in superoxide dismutase 1 (SOD1). SOD1 catalyzes the breakdown of superoxide radicals. Although decades of research have advanced our understanding of the role of SOD1 in FALS, the mechanisms behind motor neuron degeneration in FALS remain unclear. Identifying genetic suppressors will provide insight into the molecular mechanisms underlying the selective degeneration of motor neurons in SOD1 ALS models.

Methods: Using the nematode, *Caenorhabditis elegans*, we are undertaking a classical forward genetic screen to identify suppressors of glutamatergic neurodegeneration observed in a knockin SOD-1G85R model. The first ALS models of SOD1 in *C. elegans* overexpressed human SOD1 and showed increased aggregation in neurons and neuromuscular dysfunction, but no neurodegeneration was reported. Using MosSCI-mediated homologous recombination, our laboratory has generated knockin models for SOD-1G85R and other alleles. The SOD-1G85R knockin mutant animals exhibit neurodegeneration in glutamatergic neurons after stress. Using ethyl methylsulfonate, we randomly mutagenized SOD-1G85R mutant animals to induce mutations in secondary genes that could ameliorate the stress-induce neurodegeneration defect.

Results: We screened roughly 5000 F2 lines, after exposure to stress, for suppression of neurodegeneration, identified suppressor lines, and are using whole genome sequencing to identify candidate genes. Once candidate genes are identified, we will further characterize the genes by assessing cholinergic motor neuron death, neuromuscular function, and survival. Additionally, to determine if common pathways underlie neurodegeneration in FALS, we will test candidate and established ALS suppressor genes in other SOD1 and FALS models.

Discussion: Understanding the suppressors in FALS models may facilitate the development of treatments and explain some of the molecular mechanisms that lead to neurodegeneration in ALS.

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IVV-30 Profound muscular pathology in mice expressing WT and F115C mutant matrin 3 is not directly linked to motor dysfunction

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Keywords: matrin 3, transgenic mouse model

Background: Mutations in the nuclear matrix protein Matrin 3 are found in cases of both ALS and autosomal dominant distal myopathy with vocal cord and pharyngeal weakness. Previously, we generated and characterized transgenic mice expressing wild-type human Matrin 3 under the mouse prion promoter, termed PrP-MATR3^{WT}. These mice develop a phenotype of hindlimb paresis or paralysis with hindlimb and forelimb muscle atrophy. Biochemical analysis of gastrocnemius muscle from symptomatic mice revealed increased levels of products of ~120 (doublet), 118, 90, 70, and 55 kDa molecular mass that were immunoreactive with an antibody for Matrin 3. Microscopic analysis of the gastrocnemius and bicep of phenotypic mice showed a striking presence of vacuoles, nuclear chains, rounded fibers, and an increase in Matrin 3 immunoreactive internal nuclei in the fibers. Although there was no overall increase in Matrin 3 expression in the spinal cord via Western blot, there were individual cells with higher levels as well as cells with cytoplasmic immunostaining of Matrin 3 and showed increased gliosis.

Follow-up analysis: In our follow-up analysis, we have found that in addition to the gastrocnemius and biceps, distal and proximal muscles of the hindlimb (soleus, tibialis anterior, and quadriceps) and forelimb muscles (triceps, and extensor carpi radialis) display pathological features including vacuoles and rounded fibers, as well as an increase in Matrin 3 immunoreactivity in the nucleus. We have now also generated three lines of transgenic mice expressing human F115C mutant Matrin 3 under control of the mouse prion promoter, hereafter termed PrP-MATR3^{FC}. Only a few mice have developed a phenotype

up to two years of age; however, the gastrocnemius of both young and aged animals shows pathology including subsarcolemmal vacuoles.

Discussion and conclusion: This pathology appears progressive as aged mice have more profound pathology in the gastrocnemius as well. We are currently trying to understand how PrP-MATR3^{FC} mice can present with the same profound muscular changes that the PrP-MATR3^{WT} mice have, yet rarely present with a motor phenotype. Understanding the origin of this critical difference may provide critical knowledge on Matrin 3 in normal and disease biology.

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IVV-31 Disease models of ALS/FTD – a human pathological perspective

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Keywords: experimental models, TDP-43, phenotypes

Background: Amyotrophic lateral sclerosis (ALS) occurs sporadically in ~90% of patients (SALS) and almost all autopsied cases of SALS demonstrate cytoplasmic TDP-43 inclusions in the surviving lower motor neurons. In behavioural variant frontotemporal dementia (bvFTD), neuronal inclusions containing either TDP-43 or 3-repeat tau are the most prevalent. Although the genetic mutations associated with ALS and bvFTD have allowed various experimental models to be developed, the initial genetic forms identified remain the most commonly employed models to date.

Aim and method: In this study we compare the most common phenotypes identified in human ALS and bvFTD with the animal and cellular models most widely employed in preclinical and molecular studies, with the purpose of assessing the potential utility of these models in understanding pathogenesis.

Results and discussion: We demonstrate that the most commonly employed experimental models recapitulate different aspects of the diverse clinical phenotypes in ALS and bvFTD, with different models recapitulating some disease aspects, but no model faithfully recapitulating all disease aspects. This may be expected given the complexity of these syndromes in general, but it should be noted that many of the current models being pursued are driven by genetic mutations either observed in only a small minority of patients, or that are known to have divergent disease mechanisms (for example, C9orf72). If our expectation of a 'good' model is to be a perfect replica of human ALS/FTLD in the majority of cases, then all models will continue to fail in this aspect. The data presented show reasonable comparisons of feature phenotypes that we suggest could be used as surrogate readouts to layer knowledge of the complex neuronal or neuronal network dysfunction observed in patients. This would allow information to be gleaned from a variety of different yet relevant models, each of which has the capacity to capture a certain aspect of the disease, and together will enable a more complete understanding of these complex and multi-layered diseases. In summary, rethinking expectations for experimental models to fully recapitulate the most common human ALS/FTLD phenotypes will improve translation of molecular concepts that appear to have relevance.

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IVV-32 Micro-CT for non-invasive evaluation of muscle wasting in mouse models

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Keywords: muscle wasting, mouse model, imaging

Background: Much evidence indicates that the pathological process in ALS extends beyond motor neurons. Other cell types, including muscle fibers, are probably contributing to the pathology. Neuroprotective therapies that could rescue motor neurons have had a limited effect on muscle denervation and survival. It is therefore important to consider the specific effect on muscles, of potential new drugs, when developing a therapeutic approach for ALS. The evaluation of the degree of muscle atrophy in diseased mouse models is often overlooked since it requires the sacrifice of the animals for muscle examination or very high-cost instrumentation and highly qualified personnel (eg MRI). Very often behavioural tests for muscle strength evaluation are used as an outcome measure in preclinical therapeutic trials. However, these tests are easy to perform serially, but have low sensitivity in detecting early muscle changes during disease progression. Monitoring muscle loss in living animals could allow for more informative preclinical trials with a precise evaluation of therapeutic benefit with respect to muscle wasting.

Objectives: To develop a non-invasive procedure based on micro-computed tomography (micro-CT) to monitor hind limb muscle wasting in mouse models of ALS.

Methods: Mouse models: SOD1^{G93A} mice on a C57BL/6JOlaHsd background (ALS), and murine C26 colon cancer cells inoculated in 9-week-old BALB/c male mice (cancer cachexia). Micro-CT scan of hind limbs was performed with an Explore Locus micro-CT scanner (GE Healthcare) on anaesthetized mice placed prone on the micro-CT bed. The scanned images were reconstructed in 3D and analyzed using Micro View analysis software (GE Healthcare).

Results: We established the scanning procedure and the parameters to consider in the reconstructed images to calculate the index of muscle mass (IMM). We performed longitudinally a micro-CT scan of hind limbs in SOD1^{G93A} mice at presymptomatic and symptomatic stages of the disease and calculated the IMM. We found that IMM in SOD1^{G93A} mice was lower than age-matched controls very early, before symptom onset. We also detected a further decrease in IMM as disease progressed, most markedly just before disease onset. We finally examined whether there was a correlation between IMM and tibialis anterior and gastrocnemius muscle weight. We performed the same analyses in a mouse model of cancer cachexia. We found that IMM significantly correlated with both muscle weights in both mouse models.

Discussion and conclusions: We developed a fast and easy-to-conduct imaging procedure to monitor hind limb muscle mass that could be implemented in therapeutic preclinical trials but also in proof-of-principle studies to identify the onset of muscle wasting. This procedure could be widely applied to other disease models characterized by muscle wasting, to assist drug development and search for early biomarkers of muscle wasting.

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IVV-33 Modelling ALS in the visual system

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Keywords: axon, retina, cytoskeleton

Background and objectives: Axonal and synaptic degeneration are key pathological features of ALS, although the cause is yet to be fully determined. There have been challenges in developing high throughput models to determine mechanisms of degeneration *in vivo*, particularly those relating to CNS neurons. We have utilized the visual system of rodents to examine the effect of ALS relevant pathogenic mechanisms and proteins on retinal ganglion cells and their axons. This isolated system benefits from the ability to examine the effect of a somal insult on an axonal tract and terminating synapse. Retinal ganglion cells are accessed through intravitreal injection, and functional testing as well as histological examination can be performed following treatments.

Methods and results: All animal use was approved by the University of Tasmania animal ethics committee. To examine the effects of excitotoxic mechanisms we have exposed retinal ganglion cells of C57Bl/6 mice to an intravitreal injection of 10 nmoles kainic acid, which resulted in a significant and complete loss of visual function one day later ($n=6$, $p<0.05$). Immunohistological examination of whole mount retinal tissue demonstrated distinct neurofilament pathology in the non-myelinated proximal regions of axons, including large neurofilament rich swellings, endbulbs and ring-like structures, as well as increased expression of the cytoskeletal protein α-internexin, which we have reported previously in the spinal cord of a mutant SOD1 model of ALS. Microtubules were relatively spared by this treatment and remained intact in the presence of neurofilament pathology. Downstream analysis in the optic nerve cross-sections demonstrated the presence of large swollen axons and aberrant morphology in approximately 12% of axons. To examine the effects of ALS pathogenic proteins in this model, we transduced retinal ganglion cells with viral constructs encoding TDP-43, yielding stable expression of GFP-tagged TDP-43 in approximately 40% of cells, localized to the nucleus. No significant loss of visual acuity ($p>0.05$, $n=10$) was present at six weeks following transduction. Introduction of a *TDP-43* gene with a mutant nuclear localization signal resulted in mislocalization to the cytoplasm and a significant loss of visual acuity at six weeks post treatment ($p<0.05$, $n=10$).

Discussion: Our future investigations will use electron microscopy and live *ex vivo* imaging to determine the cellular effects of pathology on the axonal cytoskeleton, synapses and transport. These models will also be used in mechanistic studies of axonal and synaptic degeneration and enable therapeutic testing of protective agents. These data suggest that the visual system can act as a useful model of neurodegeneration in ALS, providing many of the benefits of *in vitro* studies including rapid screening of the effects of mutant proteins and pathological conditions on neuronal structure and function.

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IVV-34 Scientific background for developing oral levosimendan (ODM-109) for the treatment of amyotrophic lateral sclerosis

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Keywords: levosimendan, skeletal muscle function, diaphragm

Background: Levosimendan was initially developed as an IV infusion for the short-term treatment of acutely decompensated severe chronic heart failure. Currently,

orally administered levosimendan is under development for the symptomatic treatment of ALS.

Objectives: To provide scientific support for developing oral levosimendan for the treatment of ALS.

Methods: Literature review and an experimental study in an antibody induced myasthenia gravis (MG) rat model.

Results: Levosimendan is a calcium sensitizer binding selectively to troponin C. It does not increase ATP or myocardial oxygen consumption and does not cross the blood-brain barrier. Levosimendan has shown to enhance the submaximal force of fast and slow diaphragm muscle fibres (*ex vivo*), obtained from heart failure rats and patients with and without chronic obstructive pulmonary disease. This beneficial effect was also shown *in vivo* in healthy volunteers. In MG, acetylcholine receptors in the neuromuscular junction are blocked by autoantibodies, thus functionally mimicking impaired motor neuron function in ALS. In the antibody induced MG rat model, levosimendan (0.25 mg/kg) improved exercise endurance on a treadmill at two hours after a single oral dose (44 ± 66 seconds, $p = 0.06$, $n = 4$) compared to vehicle (-129 ± 46 seconds, $n = 5$). These results suggest improved skeletal muscle function in this MG model.

Discussion and conclusions: There is strong scientific evidence supporting clinical development of oral levosimendan to improve respiratory and skeletal muscle function for the symptomatic treatment of ALS.

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IVV-35 Lead identification and optimization in an *in vivo* tunicamycin assay

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Keywords: tunicamycin, UPR, GSK2606014

Background: The unfolded protein response (UPR) is an adaptive response to the accumulation of misfolded proteins in the lumen of the ER, a condition called ER stress. Prolonged ER stress activates UPR pathways that lead to apoptotic cell death and is a key contributor to numerous diseases, including amyotrophic lateral sclerosis (ALS). Drugs that target components of the UPR may be effective strategies to combat ER stress-associated pathologies. Tunicamycin is commonly used to induce ER stress and UPR activation in mammalian cell-based assays. Even though these assays are useful in identifying UPR-active compounds, they do not always translate well *in vivo*. Whole animal *in vivo* systems modeling ER stress and UPR activation, as secondary screens, may improve identification of therapeutic leads targeting these pathways.

Objectives: We sought to develop an *in vivo* tunicamycin-based assay as a secondary screen for UPR-active compounds prior to testing them in lengthy and costly ALS-specific pharmacodynamics and drug efficacy studies.

Methods: A single dose of tunicamycin (1 mg/kg, i.p.) was administered to adult, wild-type mice to induce ER stress and UPR activation. The specific PERK inhibitor GSK2606014 (18, 50 mg/kg oral gavage) was dosed two hours prior to tunicamycin challenge. Six hours post-administration the expression of 24- UPR-associated genes was measured in liver tissue via qPCR low density arrays. The liver was assayed as a surrogate tissue for the CNS to circumvent tunicamycin's inability to cross the blood-brain barrier.

Results: Tunicamycin caused widespread gene up-regulation in all three arms of the UPR pathway: PERK, Ire1, and ATF6, culminating in a 100-fold up-regulation of CHOP, a downstream target of PERK. This robust up-regulation of CHOP provides a broad dynamic window that can be used to assess the effectiveness of candidate drugs targeting the UPR. Pre-treatment with GSK2606014 inhibited the effects of tunicamycin in a dose-dependent fashion. CHOP expression was significantly reduced by 45% and 68% in the low and high dose groups, respectively. This attenuation was specific to genes downstream of PERK while gene up-regulation in the other arms was unaffected.

Discussion and conclusions: This assay produces reliable and reproducible changes in UPR gene expression that can be modulated via drug action. It can be used to assess drugs, to study disease biology in an organismal setting, and to inform the design of *in vivo* efficacy studies. One limitation is that for CNS targets, drug tissue distribution and PK parameters should be determined first. We are continuing to utilize this secondary screen to identify and optimize leads from a library of compounds targeting ER stress and UPR pathways.

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IVV-36 Development of an AAV gene therapy targeting SOD1 for the treatment of ALS: translation of delivery

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Keywords: SOD1, AAV gene therapy, RNA interference

Background: Adeno-associated viral (AAV) vectors have great potential for therapeutic gene delivery. one of the major challenges of AAV gene therapy is delivering the transgene of interest to target cells at levels that result in expression that is both safe and effective. For neurodegenerative diseases such as ALS, intrathecal (IT) administration of AAV has shown promise for providing gene transfer along the rostral-caudal axis of the CNS. Furthermore, IT dosing of AAV has been reported to provide less exposure to peripheral tissues, and reduced impact of immune responses than systemic dosing. However, efficacy of an AAV gene therapy has not been reported in a large animal model of ALS. Here, we describe a series of studies aimed at evaluating the IT delivery of an AAV gene therapy targeting SOD1 with RNAi for the treatment of canine degenerative myelopathy (DM), a naturally-occurring disease of companion dogs that is similar to some forms of human ALS.

Objectives: To select a primary miRNA (pri-miRNA) candidate targeting canine SOD1, and to evaluate IT dosing for efficacy of AAV gene therapy in dogs affected by DM.

Methods: To select a pri-miRNA candidate targeting canine SOD1, *in vitro* screens were conducted in 2 dog cell lines, and primary astrocytes from a DM-affected dog. *In vivo* screening was conducted in transgenic mice expressing human wild-type SOD1 (C57BL/6-Tg (SOD1)3Cje/J), as the miRNA target regions are conserved in human SOD1. RT-qPCR was used to quantify SOD1 mRNA knockdown.

A pilot IT study was conducted in the normal dog to evaluate the bio-distribution and cellular tropism of two different AAV vectors expressing a Hemagglutinin (HA) tag. Spinal cord and DRG tissues were evaluated by HA immunostaining.

The pharmacology/safety study in the normal dog was conducted with IT dosing of two AAV.miR-dSOD1 vectors to evaluate SOD1 suppression, precision and efficiency of pri-miRNA processing, and safety.

Results: Two candidate pri-miRNAs targeting canine SOD1 were selected based on *in vitro* and mouse studies. A pilot IT study in the normal dog confirmed that AAV vectors expressing HA tag distribute to regions of the nervous system that drive pathology in DM. Robust knockdown of canine SOD1 in these tissues with IT AAV.miR-dSOD1 was then demonstrated in the pharmacology/safety study in the normal dog. These results led to the selection of one AAV.miR-dSOD1 candidate for evaluation in companion dogs affected by DM for efficacy, which is ongoing.

Discussion and conclusions: Studies in the normal dog support the IT route of administration as appropriate for the treatment of ALS with AAV gene therapy. Evaluation of efficacy in canine DM will further inform the clinical translation of IT AAV gene therapy for the treatment of SOD1-ALS.

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