IVV-01: Age-associated regressive changes in the neuromuscular system of mice

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Live Poster Session A, December 9, 2020, 5:10 PM - 5:50 PM

Background:
Sarcopenia is an aging-associated condition characterized by a decline in skeletal muscle mass, strength and function (1). The cellular and molecular mechanisms underlying sarcopenia are controversial and poorly understood (2-3), hampering the development of effective therapeutic interventions.
Objectives:
To characterize the aging-related structural and molecular changes occurring simultaneously in distinct components of the neuromuscular system, including: motoneurons (MNs), glia, motor nerves, neuromuscular junctions (NMJs) and different types of skeletal muscles.
Methods:
Motor behavioral and electrophysiological tests, as well as histological and immunocytochemical procedures were carried out. Young, adult, middle-aged, and old (1, 4, 14, and 24–30 months old, respectively) C57BL/6J mice were used.
Results:
Aging was not accompanied by a significant loss of spinal MNs, although a proportion (~15%) of them in old mice exhibited an abnormally dark appearance. Dark MNs were also observed in adult (~9%) and young (~4%) animals. Morphological alterations in motor axons were already observed in adulthood but substantially increased with age. Old MNs were depleted of cholinergic and glutamatergic inputs (~40% and ~45%, respectively). Prominent microgliosis and astrogliosis [~93% and ~100% increase vs. adults, respectively] were found in old spinal cords, with increased density of pro-inflammatory microglial and astroglial phenotypes (25-fold and 4-fold increase, respectively). Aging resulted in significant reductions in the nerve conduction velocity and the compound muscle action potential amplitude (~30%, vs. adults) in old distal plantar muscles. Compared with adult muscles, old muscles exhibited significantly higher numbers of both denervated and polyinnervated NMJs, changes in fiber type composition, higher proportion of fibers showing central nuclei and lipofuscin aggregates, depletion of satellite cells, and augmented expression of different molecules related to development, plasticity, and maintenance of neuromuscular junctions, including calcitonin gene-related peptide, growth associated protein 43, agrin, fibroblast growth factor binding protein 1, and transforming growth factor-β1. Overall, these alterations occurred at varying degrees in all the muscles analyzed, with no correlation between the age-related changes observed and myofiber type composition or muscle topography.
Discussion:
Our data provide a global view of age-associated changes in the neuromuscular system of mice, some of them previously envisaged as controversial when different models and partial aspects of the aging process were assessed. Our results suggest that during aging, some MNs undergo early deleterious changes, which may not lead to MN death. Age-related MN dysfunction could be responsible for structural and molecular alterations in motor axons, MNJs, and skeletal muscles found in senescence.
References:
1 Larsson L et al. (2019). Physiol Rev; 99:427-511.
2 Berger MJ et al. (2010). Interdiscip Top Gerontol; 37:94-114.
3 Chopek JW et al. (2010). Mech Ageing Dev; 131:650-659.
Acknowledgements:
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IVV-02: ALS gene, C9orf72, loss of function Zebrafish model shows motor and synaptic defects

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects motoneurons causing muscular atrophy, paralysis and ultimately to death. To this day, no curative treatment exists. Understanding the physiopathological mechanisms will help develop new efficient treatments. In 2011, an expansion of a repetition of a hexanucleotide (GGGGCC) in the first intronic region of the C9orf72 gene has been discovered as the first genetic cause of ALS. To investigate the role of C9orf72 loss of function in ALS, we used synthetic micro-RNAs to specifically target the zebrafish C9orf72 gene (C9-miRNA) and have developed a stable zebrafish C9-miRNA line with reduced expression of C9orf72. Upon loss of function of C9orf72, we observed that zebrafish C9-miRNA mutants display severe motor deficits starting at 6 days postfertilization (6 dpf) and a majority die premature as of 15 dpf. Analysis of the neuromuscular junctions using specific presynaptic and postsynaptic markers SV2 and alpha-bungarotoxin respectively, revealed a significant decrease in the number of synaptic contacts in the C9-miRNA mutant line at 6 dpf correlating with a decreased synaptic vesicles turnover. Electrophysiology recordings using patch clamp technique on muscle fibres showed a decrease of amplitude and frequency of the spontaneous miniature end plate currents, which suggests a decrease number of presynaptic endings. Also, TDP-43 has been shown to aggregates at 6dpf in our C9-miRNA. Among the few fishes that survived till adulthood, we observed a significant motoneuron and muscle atrophy. Altogether, our zebrafish C9-miRNA replicates aspects of ALS and showed that C9orf72 has a role in the synaptic transmission at the NMJ.

IVV-03: BDNF-regulation of in vivo axonal transport is selectively impaired in fast motor neurons in SOD1G93A mice

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Live Poster Session A, December 9, 2020, 5:10 PM - 5:50 PM

Background:
Axonal transport ensures long-range, bidirectional delivery of essential cargoes between proximal and distal compartments of neurons and is needed for neuronal development, function and survival. SOD1G93A mice show in vivo deficits in axonal transport pre-symptomatically suggesting that impairment contributes to disease. α-motor neurons (MNs) are defined, in part, by the type of muscle fibre they innervate, and in SOD1G93A mice, fast-twitch muscles are more vulnerable than slow-twitch muscles. As the influence of a) α-MN subtype, and b) brain-derived neurotrophic factor (BDNF), on axonal transport is currently unknown, determining the mechanisms that regulate BDNF-signalling in MN subsets, and variations in pathology, will reveal novel clues about selective MN vulnerability in ALS.

Objectives:
1) Evaluate basal axonal transport dynamics in different muscle subtypes; and whether transport is influenced by BDNF stimulation.
2) Characterise the levels of BDNF and its receptors in skeletal muscles and at the NMJ.
3) Assess alterations in SOD1G93A pathology.

Methods:
Axonal transport of signalling endosomes was visualised in vivo with intramuscular injections of a fluorescently-labelled atoxic fragment of tetanus neurotoxin (HcT). HcT was delivered into the tibialis anterior (TA), lateral gastrocnemius (LG) or soleus muscles of wild-type (WT) and SOD1G93A mice, with or without 25 ng of recombinant BDNF. 4-6 hours post-injection, sciatic nerves were exposed in live, anaesthetised animals, and imaged using time-lapse confocal-microscopy at 37°C. Retrogradely transported, HcT-labelled signalling endosomes within single axons were tracked using the TrackMate plugin (FIJI/ImageJ). BDNF, TrkB full-length (TrkB.FL), TrkB truncated (TrkB.T1) and p75NTR levels in basal and diseased muscle were quantified by western blot. NMJ TrkB- and p75NTR -receptor content was assessed using immunohistochemistry in teased muscle-fibres and quantified using FIJI.

Results:
Basal axonal transport analysis reveals that signalling endosomes velocities are similar between all MN/muscle subgroups. BDNF-stimulation significantly enhanced axonal transport dynamics in fast MNs (FMNs) innervating TA and LG, but not slow MNs innervating soleus in WT mice. However, in SOD1G93A mice axonal transport was only significantly impaired in FMNs innervating TA. Moreover, the BDNF-mediated enhancement of axonal transport in WT mice was lost in SOD1G93A mice selectively in FMNs innervating TA and LG. Despite basal differences of BDNF, TrkB.T1 and p75NTR expression levels between TA and soleus muscles, no changes were observed during disease. Lastly, there were no pathological variances of TrkB or p75NTR receptor expression levels in SOD1G93A TA and soleus NMJs.

Discussion and Conclusions:
These data indicate that different MN/muscle subgroups have distinct axonal transport features and are differentially afflicted in SOD1G93A mice.

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Wellcome Trust Senior Investigator Award (GS; 107116/Z/15/Z), MRC Career Development Award (JNS: MR/S006990/1) and Human Frontier Science Program Long-Term Fellowship (SS; LT000220/2017-L).

IVV-04: Changes in the expression of the C-bouton-specific Y172-related antigen in association with motoneuron pathology

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Live Poster Session A, December 9, 2020, 5:10 PM - 5:50 PM

Background:
C-boutons are cholinergic inputs to motoneurons (MNs) that modulate their excitation state, which is essential to drive motor behavior. Alterations in C-boutons appear to play an important role in MN pathology, particularly in ALS and spinal muscular atrophy (SMA)[1]. During an immunocytochemical study on the role of c-Jun in MNs with a monoclonal (clone Y172) antibody against phospho-c-Jun (serine [Ser]63), we observed an unexpected labeling closely associated to C-boutons[2].

Objectives:
To obtain new insights into the role of Y172-related antigen in C-boutons and their changes in MN pathology.

Methods:
CD1 mice, and mouse models of ALS (SOD1G93A) and SMA (Smn2B/-) were used. Animal experimentation procedures were previously evaluated and approved by the Committee for Animal Care and Use of the University of Lleida. Cultured MNs of CD1 mice were used as an in vitro model. Immunocytochemical procedures were carried out and Z-stack confocal images from 10 to 15 randomly selected MNs per condition and animal (3-4 animals) were analyzed.

Results:
In adult spinal cord, MNs displayed strong Y172 immunostaining in cytoplasmic structures closely associated with C-boutons (>90%), but not with other nerve afferent types contacting MNs (<6%). By ultrastructural analysis, cytoplasmic Y172 immunostaining was selectively located at the subsurface cistern of C-boutons. The analysis of Y172 immunoreactivity in injured MNs after peripheral nerve transection, and in SOD1G93A and Smn2B/- mice, revealed a significant depletion of cytoplasmic immunostaining at advanced stages (~65% $p<0.001$ vs. contralateral; ~50% $p<0.001$ vs. WT; ~75% $p<0.01$ vs. WT, respectively), which preceded the C-bouton loss occurring in these paradigms. RNA interference experiments to knock down c-Jun in vitro by using different shRNA constructs resulted in a dramatic decrease in nuclear Y172 immunostaining in MNs without any reduction in the density of cytoplasmic Y172-positive profiles. Studies in skeletal muscles revealed that Y172-immunoreactivity was also present in neuromuscular junctions (NMJs).

Discussion:
We show here a novel unidentified molecular component of the C-bouton organization, which expression is lost in damaged MNs even before the occurrence of cholinergic deafferentation. The presence of Y172 in NMJs suggests that this protein might be axonally transported between MN soma and muscle. Our results lay the foundation for further studies aimed at identifying the Y172-related protein and determining its role in the context of the development, maintenance, plasticity and pathology of both C-boutons and NMJs.

References:
1.Witts et al., (2014). J Anat, 224, 52-60.
2.Gatius et al., (2020). Front Cell Neurosci, 13, 582.

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IVV-05: Correlation of FUS phase separation with toxicity in Drosophila models of ALS/FTD

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Live Poster Session A, December 9, 2020, 5:10 PM - 5:50 PM

Background:
FUS is a DNA/RNA binding protein that is mutated in rare familial forms of ALS, and additionally aggregates in approximately 10% of cases of frontotemporal dementia. We have previously modelled FUS-opathies in Drosophila by overexpressing either wild-type or ALS-mutation bearing human FUS (Bogaert et al. 2018). Using a domain deletion approach, we have demonstrated that the tyrosine-rich QGSY domain and the arginine-rich RGG domains act synergistically to induce toxicity, potentially acting via arginine-tyrosine cation-pi interactions.

Results and Future Objectives:
We have now generated new transgenic Drosophila lines with amino acid substitutions in FUS (previously described in Wang et al. 2018), which inhibit the liquid-liquid phase separation (LLPS) of FUS by modifying the cation-pi interaction, or alter the condensate properties of FUS. Consistent with LLPS dependent toxicity, substitution of the RGG domain arginine residues to either lysine or glycine (R to K, R to G) or QGSY domain tyrosine residues to phenylalanine or serine (Y to F, 10Y to S, 18Y to S) decreases the toxicity of overexpressed FUS. Additionally, substitutions within the QGSY domain that increase its conformational flexibility also reduce toxicity (Q to A, Q to G), whilst substitutions predicted to decrease conformational flexibility (G to A) enhance toxicity. Interestingly, substitutions resulting in lower toxicity were generally associated with lower FUS protein abundance, suggesting that LLPS protects FUS from clearance in neurons, potentially by promoting its aggregation.

To determine the mechanisms by which FUS becomes toxic to neurons in Drosophila, we performed a genome-wide unbiased deficiency screen in flies expressing R521G mutant FUS in motor neurons. Amongst other hits, one of the candidates we identified is Qkr54b (human ortholog Sam68), the loss of which rescues both toxicity and neuronal loss in Drosophila expressing FUS. Using CRISPR/Cas9 we have generated a knockout of Qkr54b and have performed characterisation of neuronal phenotypes related to its loss of function. Using overexpression in human neuroblastoma cell lines, we observed that Qkr54b forms polyA mRNA and FUS-positive aggregates. We are currently developing tools to determine whether Qkr54b undergoes liquid-liquid phase separation with FUS, and the potential mechanism(s) mediating this.

Finally, in order to further explore whether human FUS partitions into neuronal stress granules in vivo as a site of LLPS, we have used CRISPR/Cas9 to tag the endogenous Drosophila G3BP1 (Rin) with either mCherry or V5 tags. We aim to explore whether stress granules accumulate in neurons during Drosophila lifespan, and whether these stress granules recruit FUS leading to its aggregation.

References:
- Bogaert et al. (2018), Cell Reports, 24(3):529-537.e4
- Wang et al. (2018), Cell, 174(3):688-699.e16

IVV-06: Effects of C9orf72 haploinsufficiency on TDP-43 pathology in ALS

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Live Poster Session A, December 9, 2020, 5:10 PM - 5:50 PM

Background:
Neuronal cytoplasmic inclusions of abnormally phosphorylated TAR DNA-binding protein 43 (TDP-43) is a pathological hallmark of ALS and frontotemporal lobar degeneration (FTLD). In recent years, several studies have found a strong association between autophagy and ALS; many causative genes implicated in ALS have functions in autophagy. This includes C9orf72, which encodes for a DENN domain containing protein with reported roles in autophagy. Hexanucleotide repeat expansions in a non-coding region of C9orf72 is the most common genetic cause of ALS/FTLD and is associated with a loss of function mechanism caused through haploinsufficiency, leading to reduced levels of C9orf72 protein. It is our hypothesis that loss of C9orf72 could lead to autophagic deficits promoting TDP-43 pathology.

Objective:
To investigate if loss of C9orf72 promotes TDP-43 pathology in vivo.

Methods:
The effects of C9orf72 haploinsufficiency on promoting TDP-43 pathology was investigated in C9orf72 knockout (C9KO) and wild type (WT) mouse littermates expressing two pathological variants of TDP-43, EGFP-TDP-35 or EGFP-TDP-25, delivered through neonatal intracerebroventricular injection of rAAV9 under control of the hSYN1 promotor.

Results:
Widespread expression of virally delivered EGFP, EGFP-TDP-35 and EGFP-TDP-25 was present throughout the brain of 8-month-old WT and C9KO mice. Rare cytoplasmic aggregates of EGFP-TDP-35 were present in the cortex of WT mice that occasionally co-labeled with autophagy marker p62 and phosphorylated TDP-43 antibody (pTDP-43). Although no apparent increase in the abundance of neuronal cytoplasmic aggregates of EGFP-TDP-35 in C9KO mice was seen, there was a marked increase in p62 labeled cytoplasmic aggregates as well as increased pTDP-43 labeling. Expression of EGFP-TDP-25 gave abundant and striking neuronal cytoplasmic inclusions in the cortex and olfactory bulb of WT mice, which were significantly more abundant in C9KO mice, and correlated with increased p62 and pTDP-43 labeling.

Discussion:
Here we have demonstrated that viral mediated expression of EGFP-TDP-35 and EGFP-TDP-25 effectively produces mouse models in which to study TDP-43 cytoplasmic aggregation. Importantly, we show that loss of C9orf72 increases the abundance of cytoplasmic EGFP-TDP-25 aggregates, as well elevated levels of p62 and pTDP-43 aggregate labeling in both EGFP-TDP-35 and EGFP-TDP-25 mice. These findings support our hypothesis that loss of C9orf72 function promotes TDP-43 pathology, with the increase in p62 labeling in the context of C9orf72 deficiency suggesting that this may be through a defect in autophagy.

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IVV-07: How the partial deletion of mGluR5 affects the pro- and anti-inflammatory features of microglia during ALS progression in SOD1G93A mice

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Live Poster Session A, December 9, 2020, 5:10 PM - 5:50 PM

Introduction:
Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by the selective death of upper and lower motor neurons (MNs). The etiology of the disease is not completely understood, and among the several mechanisms that concur to the pathogenesis and progression of the disease, glutamate (Glu)-mediated excitotoxicity and neural inflammation play a pivotal role. ALS is also a multicellular disease, being astrocytes and microglia involved in neurodegeration, acquiring a reactive phenotype during disease progression. Metabotropic Glu receptors 5 (mGluR5) are deeply involved in ALS, even if the importance in the different cellular populations has not been investigated. In our previous studies we generated double mutant mice carrying the SOD1G93A mutation and the mGluR5 partial deletion (SOD1G93AmGluR5+/-). These mice displayed a delay of the pathology onset, an amelioration on survival probability and an improvement in clinical signs.

The aim of this study was to investigate the effect of the partial deletion of mGluR5 in microglia cells isolated from mutant mice at different time points, that represent respectively pre symptomatic stages, disease onset and the end of life. Several metabolic analysis on the different animal models used are carrying on.

Methods:
Microglia cells were acutely isolated through a discontinuos Percoll gradient from motor cortex and spinal cord of WT, SOD1G93A and SOD1G93AmGluR5+/- mice at three different time points during disease progression. TMEM119-positive cells were analyzed by flow cytometry. The pro-inflammatory M1 and the anti-inflammatory M2 phenotypes were detected and confocal analysis were performed to demonstrate the existence of mGluR5 on microglia. We performed oximetric and luminometric analysis to evaluate the oxygen consumption and ATP synthesis.

Results:
The M1/M2 ratio augmented in the spinal cord of SOD1G93A, but it is statistically significant in SOD1G93AmGluR5+/- mice at the late symptomatic phase of the disease only, while did not change in microglia derived from motor cortex. Our results also highlight a bioenergetic impairment in microglia derived from 120 day-old SOD1G93A mice respect to age matched controls, that is partially restored in SOD1G93AmGluR5+/- mice.

Conclusions:
The reduction of mGluR5 in SOD1G93AmGluR5+/- mice forces spinal cord isolated from microglia toward a more pro-inflammatory phenotype, at least at the late stage of disease progression.

IVV-08: Humanised and physiological mouse models of FUS-ALS

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Live Poster Session A, December 9, 2020, 5:10 PM - 5:50 PM

By working with genetic mouse models of human disease we can unravel disease mechanisms and start to produce specific therapies to modulate neurodegeneration. However, most transgenic models overexpress proteins of interest, and may produce phenotypes unrelated to the disease itself. Here, we present work on two new FUS Knock-in models. (1) FUS-delta14 homozygotes: Previously, we have reported that heterozygous FUS-Delta14 knock-in mice, harbouring a FUS-ALS splice-site patient mutation, display progressive, late onset motor neuron degeneration. Here we present data on homozygous FUS-delta14 mice, which present with motor impairment from a young age with a progressive loss of motor neurons beginning at 1 month of age, together with a reduction in functional motor units, reduction in muscle strength, and an increase in slow twitch fibres in TA muscle. (2) Humanised FUS (hFUS) knock-in mice: In order to produce better physiological models that express human mutant genes at physiological levels, we are developing new humanised knock-in strains, including the first genomically humanised FUS knock in mice, which express human FUS protein from the endogenous Fus locus, spanning from the ATG start codon through to the 3' UTR, including all intervening exons and introns. We present here phenotyping analysis of humanised FUS homozygous mice, showing that human FUS functionally replaces the essential mouse Fus gene and that these mice are grossly normal, including at the global transcriptome level. We have further introduced ALS mutations into the humanised allele via CRISPR/Cas9, and present early phenotyping work on hFUS-P525L mice.

IVV-09: Identifying Neuregulin-mediated microglial activation in ALS

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Live Poster Session A, December 9, 2020, 5:10 PM - 5:50 PM

Background: To understand disease progression in human ALS patients, we compared transcriptional profiles of laser captured motor neurons between more and less affected human ALS spinal cord regions. Using a gene clustering approach, we identified multiple subtypes of both inflammatory cells (microglia) and motor neurons (1). In previous work, we have implicated NRG1 signaling in both human ALS and the SOD1 G93A mouse model showing that NRG1 receptors are constitutively activated on microglia (2,3) and that blocking NRG1 signaling with a potent, targeted antagonist slows disease onset and spread in mice (4). These findings raise the hypothesis that NRG1 release from degenerating motor neurons activates surrounding microglia thus promoting the disease progression in the central nervous system. Objectives: Identify the role of NRG1 signaling in human ALS and evaluate the therapeutic potential of targeting NRG1-signaling on microglia using a new animal model. Methods: Identifying NRG1 and NRG1 receptors in multiple subtypes of microglia and motor neurons from human ALS samples using a gene clustering approach. Generating a novel inducible transgenic mice to block NRG1 receptor activation to test whether NRG1 promotes microglial activation leading to motor neuron degeneration. Results: In human ALS, NRG1 splice forms are differentially expressed as a function of motor neuron degeneration while NRG1 receptors are expressed on a subtype of microglia associated with degenerating motor neurons. NRG1 induces pro-inflammatory cytokine expression and promotes phagocytic activity in cultured microglia. Consistently with our therapeutic approaches, we selectively delete NRG1 signaling in microglia from SOD1 mice in vivo using an inducible dominant-negative (DN) NRG1 receptor (erbB4) system. DN-erbB4Cherry expresses on cells with microglial morphology and co-localizes with ErbB4 in the brain from the DN-erbB4cherry:CX3CR1eyfpCreERT2 double Tg mice. Bodyweight, disease onset, and progression, animal survival as well as pathological changes in the DN-erbB4cherry:CX3CR1eyfpCreERT2:SOD1 G93A triple Tg mice are on the way. Discussion/Conclusions: These studies provide mechanistic insights into how NRG1 signaling on microglia may lead to disease progression and suggest blocking NRG1 as a novel therapeutic for human ALS.

References:

1. Dachet F, Liu J, Ravits J, Song F. Predicting disease stage specific spinal motor neurons and glia in sporadic ALS patients. Neurobiol Dis. 2019; 130:104523.
2. Song F, Chiang P, Wang J, Ravits J, Loeb JA. Aberrant neuregulin 1 signaling in ALS. J Neuropathol Exp Neurol. 2012. 71:104.
3. Song F, Chiang P, Ravits J, Loeb JA. Activation of microglial neuregulin1 signaling in the corticospinal tracts of ALS patients with upper motor neuron signs. Amyotroph Lateral Scler Frontotemporal Degener. 2014. 15:77.
4. Liu J, Allender E, Wang J, Simpson EH, Loeb JA, Song F. Slowing disease progression in the SOD1 mouse model of ALS by blocking neuregulin-induced microglial activation. Neurobiol. Dis. 2018; 111:118.

IVV-10: Mitochondria of upper motor neurons with TDP-43 pathology undergo mitoautophagy, a unique self-destructive path, very early in ALS

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Background:
Upper motor neuron degeneration is a hallmark characteristic of numerous neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS). Understanding the cellular and molecular mechanisms responsible for their progressive degeneration due to different underlying causes is required to build effective treatment strategies. Mitochondria are one of the key organelles at the heart of many of the cellular functions responsible for motor neuron health. Mitochondrial dysfunction is thus one of the converging paths for many neurodegenerative diseases. Recent evidence reveals the presence of mitochondrial defects in corticospinal motor neurons (CSMN) with TDP-43 pathology. However, how early these defects begin to occur and whether they are shared among CSMN that become vulnerable to disease due to different underlying causes are not known.

Objective:
Mitochondrial defects occur very early in the disease, and especially in CSMN defective mitochondria contributes to neuronal vulnerability.

Methods:
Since structural integrity is required for proper mitochondrial function, we used immuno-coupled electron microscopy for a detailed surveillance of mitochondria in CSMN and other non-CSMN cells at P15 (post-natal day 15), –a very early age in mice without any sign of cellular neurodegeneration. We have investigated ultrastructure of mitochondria in CSMN and non-CSMN cells from hSOD1G93A, prpTDP-43A315T, and PFNG118V mouse models of ALS.

Results:
We find mitochondria to undergo mitoautophagy, a systematic and novel path of self-destruction mainly in the CSMN of prpTDP-43A315T mice, even at P15. Even though having defective mitochondria is one common cellular pathology the extent and the type of pathology differs among CSMN that become diseased due to different underlying cause. There are signs of degeneration also in CSMN of PFNG118V mice, albeit to a lesser extent, and the mitochondria in CSMN of hSOD1G93A mice are extensively expanded in size, but do not undergo mitoautophagy.

Discussion/Conclusions:
Our findings reveal mitochondrial defects to occur very early especially in CSMN with TDP-43 pathology. Even though problems with mitochondria can be considered as a common cause, there are still distinct and important differences between CSMN that become diseased due to different underlying causes. Understanding such differences will help develop more targeted and effective treatment strategies.

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IVV-11: Premotor interneuron networks innervating fast-twitch fatigable and slow-twitch fatigable-resistant motor neurons and its implications for ALS

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Live Poster Session A, December 9, 2020, 5:10 PM - 5:50 PM

Abstract:
A signature of Amyotrophic Lateral Sclerosis (ALS) is that motor neurons innervating fast-twitch fatigable muscles are more vulnerable compared to those innervating slow-twitch fatigable resistant muscles (1,2). However, the reasons for this differential vulnerability remain unknown. Here, we focus on identifying the complex spinal interneuron circuits that orchestrate activity of the different motor neuron populations. Using mono-synaptically-restricted trans-synaptic labelling, we are able to target specific groups of fast and slow motor neurons and visualize their specific interneuron inputs in the spinal cord (3). By combining this labelling technique with CLARITY tissue clearing (4), we show a differential spatial localization of premotor interneurons, with interneurons innervating fast motor neurons localizing at a dorso-medial position compared to the more ventro-lateral localization of interneurons innervating slow motor neurons. Moreover, using novel in situ sequencing technique (5) and markers specifically expressed in different excitatory and inhibitory spinal interneuron subpopulations, we can further identify the visualized interneurons and determine their molecular identity. For this purpose, we make use of a fluorescent Nissl staining in combination with machine learning-based methods –more specifically convolutional neural network (6)– to identify both nuclei and neuronal cell boundaries within the tissue, and allocate the specific markers detected by in situ sequencing to the fluorescently labelled neurons. Co-localisation of these markers by registration method (7) allows us to assign all premotor inputs of fast and slow motor neurons to known subpopulations of interneurons. Given our previous work indicates that the interneuron-motor neuron circuitry is indeed affected in ALS (8), once these complex neural networks have been decoded future directions will focus on elucidating how are they affected and which cell types and underlying molecular mechanisms contribute to disease pathology. All in all, our results provide new knowledge about motor control circuitry while contributing to the advance of new analysis methods for in situ sequencing data, but also open a unique pathway towards the development of novel therapeutic targets for ALS.

References:
1) Pun S et al. (2006). Nat Neurosci 9(3):408-19
2) Roselli F & Caroni P (2015). Neuron 85(5):901-10
3) Reardon TR et al. (2016). Neuron 89(4):711-724
4) Tomer R et al. (2014). Nat Protocols 9(7): 1682-1697
5) Ke R et al. (2013). Nat Methods 10(9): 857-860
6) Ronneberger O et al. (2015). MICCAI 2015: 234-241
7) Butz T & Thiran JP (2001). MICCAI 2001: 549-556
8) Allodi I et al. (2020 preprint). https://doi.org/10.1101/2020.06.23.166389

Acknowledgements:
This work was supported by the Lundbeck Foundation, the Novo Nordisk Foundation, the Louis-Hansen Foundation, the Björklund Foundation, the A.P. Møller Foundation, and the Faculty of Health and Medical Sciences at UCPH.

IVV-12: Zebrafish knock-in models of commonly found TARDBP (TDP-43) mutations display a robust degenerative motor phenotype

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Live Poster Session A, December 9, 2020, 5:10 PM - 5:50 PM

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease that is characterized by loss of upper and lower motor neurons which eventually leads to paralysis and death within 2-5 years of diagnosis. A pathological hallmark of the disease is the cytoplasmic mislocalization of the nuclear RNA binding protein TAR-DNA Binding Protein of 43 kDa (TDP-43) in motor neurons in ~97% of all ALS cases. Mutations in TARDBP, encoding TDP-43, are associated with a small percentage of ALS cases, and among these are the mutations encoding the A382T (the most commonly found variant) and G348C TDP-43 variants. By using the CRISPR/Cas9 mutagenic system, we have developed knock-in zebrafish lines carrying point mutations that encode these predicted A379T and G347C variants in Tdp-43, which are analogous to the human A382T and G348C variants, respectively. tardbpA379T/WT and tardbpA379T/A379T zebrafish were found to have a reduced lifespan compared to their wild-type siblings. As adults, the heterozygous and homozygous mutant fish develop a degenerative motor phenotype that can be seen around 2 years of age in both a free swim assay and a swim tunnel. This phenotype occurs around the time that the loss of large spinal motor neurons is observed. Furthermore, while Tdp-43 expression levels in the brain were similar between wild-type and mutant 1-year old zebrafish, a compensatory splice variant (tardbpl_tv1) that arises from a tardbp paralogue present in the zebrafish genome (tardbpl) was significantly increased in tardbpA379T/A379T and tardbpG347C/G347C brains. At this pre-symptomatic age, we also performed RNA-sequencing of spinal cord tissue from homozygous mutants and found a strong upregulation of transcripts involved in the immune response and inflammation in both genotypes. Lastly, we found that ablation of tardbpl expression led to a ~2-fold increase in Tdp-43, which we hypothesize will lead to a more severe phenotype in animals that harbour tardbp mutations while also missing this zebrafish-specific paralogue. We believe these animal models more accurately represent the human genetic state of the disease and propose that they could serve as both a valuable tool to investigate cellular defects arising in ALS and the development of precision therapeutics.

IVV-13: Humanised and physiological mouse models of SOD1 and C9orf72 ALS

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Live Poster Session B, December 10, 2020, 5:10 PM - 5:50 PM

Modelling late onset neurodegenerative disease is challenging, and improved models are needed to more faithfully recapitulate human pathology and develop treatment strategies. The key long-term focus of our lab is to genetically engineer new mouse models of Amyotrophic Lateral Sclerosis (ALS) by genomic humanisation at the endogenous locus (control mice) as well as introducing patient mutations into the humanised alleles (mutant mice). These physiological mouse models will be analysed using a range of phenotyping methods, which will answer key questions surrounding early disease pathomechanisms in ALS. Our physiological mouse models will also serve as more accurate models to test future therapeutics.

Among the familial ALS etiologies, mutations in C9orf72 account for 30-40% cases, followed by SOD1 accounting for 15-20% cases. Here we present ongoing work on the generation and characterization of humanised C9orf72 and SOD1 mouse models.

To humanise the mouse Sod1 allele, a targeting construct carrying full length SOD1 was introduced to mouse ES cells by homologous recombination. Cohorts carrying fully humanised SOD1 (control mice) are being phenotyped currently to assess the impact of genomic humanisation. In addition, we have introduced the A4V mutation into the humanised SOD1 allele via CRISPR/Cas9 editing in zygotes. Breeding is currently ongoing to generate cohorts carrying fully humanised SOD1 with the A4V mutation, which will then undergo thorough phenotyping.

To generate mouse models carrying humanised C9orf72, two targeting constructs have been engineered. The first harbours wild type full-length human C9orf72 and was generated via recombineering in BAC vectors (control mice). The second carries full length human C9orf72 plus ~1000 GGGGCC repeats in intron 1 (mutant mice), as seen in human C9orf72-ALS patients, which was generated via a multistep process utilising novel CRISPR/Cas9 cloning and recursive cloning techniques. These targeting constructs are currently being used to genetically modify mouse ES cells.

We propose that these genetically humanised mouse models of SOD1 and C9orf72 ALS will provide new insights into how mutant human proteins can trigger ALS, and could prove to be a useful model for preclinical ALS drug development.

IVV-14: Inclusion formation and toxicity of the ALS protein Matrin3 is modified by molecular chaperones

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Live Poster Session B, December 10, 2020, 5:10 PM - 5:50 PM

The Matrin3 protein encoded by the MATR3 gene has been implicated in amyotrophic lateral sclerosis (ALS) (1-3). Biochemical and pathological studies have shown that Matrin3 forms neuronal cytoplasmic and nuclear inclusions in ALS-affected neurons (1-3). Additionally, over 30 heterozygous missense mutation in MATR3 have been identified in a number of familial ALS (fALS) and sporadic ALS (sALS) cases (1-3). Little is known about the normal biological function of Matrin3 or how it contributes to ALS pathogenesis. To further explore Matrin3 we have established and characterized a yeast model. We demonstrate that wild type Matrin3 and the ALS-associated variant F115C are toxic when overexpressed and form inclusions. Additionally, through unbiased screening using the yeast deletion library, we have identified Ydj1, an Hsp40 protein, as a potent modifier of Matrin3 cytotoxicity. Our functional characterization uncovers substantial modification of Matrin3 toxicity and inclusion formation by Hsp40s and Hsp90 and its co-chaperones. Our study thus demonstrates for the first time how specific branches of cellular protein quality control regulate the misfolding and toxicity of Matrin3.

References:

1. JOHNSON, J. O., PIORO, E. P., BOEHRINGER, A., CHIA, R., FEIT, H., RENTON, A. E., PLINER, H. A., ABRAMZON, Y., MARANGI, G., WINBORN, B. J., GIBBS, J. R., NALLS, M. A., MORGAN, S., SHOAI, M., HARDY, J., PITTMAN, A., ORRELL, R. W., MALASPINA, A., SIDLE, K. C., FRATTA, P., HARMS, M. B., BALOH, R. H., PESTRONK, A., WEIHL, C. C., ROGAEVA, E., ZINMAN, L., DRORY, V. E., BORGHERO, G., MORA, G., CALVO, A., ROTHSTEIN, J. D., DREPPER, C., SENDTNER, M., SINGLETON, A. B., TAYLOR, J. P., COOKSON, M. R., RESTAGNO, G., SABATELLI, M., BOWSER, R., CHIO, A. & TRAYNOR, B. J. 2014. Mutations in the Matrin 3 gene cause familial amyotrophic lateral sclerosis. Nat Neurosci, 17, 664-666.
2. BOEHRINGER, A., GARCIA-MANSFIELD, K., SINGH, G., BAKKAR, N., PIRROTTE, P. & BOWSER, R. 2017. ALS Associated Mutations in Matrin 3 Alter Protein-Protein Interactions and Impede mRNA Nuclear Export. Sci Rep, 7, 14529.
3. SENDEREK, J., GARVEY, S. M., KRIEGER, M., GUERGUELTCHEVA, V., URTIZBEREA, A., ROOS, A., ELBRACHT, M., STENDEL, C., TOURNEV, I., MIHAILOVA, V., FEIT, H., TRAMONTE, J., HEDERA, P., CROOKS, K., BERGMANN, C., RUDNIK-SCHÖNEBORN, S., ZERRES, K., LOCHMÜLLER, H., SEBOUN, E., WEIS, J., BECKMANN, J. S., HAUSER, M. A. & JACKSON, C. E. 2009. Autosomal-dominant distal myopathy associated with a recurrent missense mutation in the gene encoding the nuclear matrix protein, matrin 3. Am J Hum Genet, 84, 511-8.

IVV-15: Increasing endocannabinoid levels by FAAH inhibition is neuroprotective in a model of TDP-43 dependent frontotemporal dementia

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Live Poster Session B, December 10, 2020, 5:10 PM - 5:50 PM

Frontotemporal dementia (FTD) is a heterogeneous group of early-onset and progressive neurological disorders, characterized by neuronal degeneration in the frontal and temporal lobes, which alters cognition, personality, social behavior and language. Approximately 65% of FTD patients present TDP-43 aggregates, a protein involved in the regulation of RNA stability, transport and transcription. This protein has been also associated with amyotrophic lateral sclerosis (ALS), so nowadays, both pathologies are considered a continuous clinical spectrum with common pathogenic mechanisms and without an effective treatment. Our group has recently provided preclinical evidence that the modulation of the endocannabinoid signaling (ECS), an endogenous neuromodulatory system, could delay the progression of TDP-43-dependent ALS due to its capability to interfere in multiple pathogenic mechanisms involved in this disease.

We are now interested in investigating whether the modulation of the ECS may be also useful in FTD. To this end, we used mice that overexpress TDP-43 exclusively in the forebrain under the control of α-CaMKII promoter, then showing marked symptoms of dementia from day PND60 (provided by James C. Shen, Tsai et.al, 2010. First data in our laboratory with these mice revealed a decrease in the hydrolyzing enzyme FAAH in the prefrontal cortex (mPFC) and the hippocampus, and an increase in the synthesizing enzyme NAPE-PLD in the hippocampus. Both responses may be interpreted as an endogenous protective response directed to elevate endocannabinoids levels in these structures, in particular anandamide.

In the present study, we wanted to confirm this hypothesis, using pharmacological inactivation of FAAH enzyme with the selective inhibitor URB597, which is well-demonstrated that results in elevated anandamide levels. The drug was administered via i.p at a dose of 0.2 mg/kg on alternate days from PND45 (pre-symptomatic stage) to PND90 (symptomatic stage). Our data revealed that the inhibition of FAAH enzyme was associated with a delay in the appearance of cognitive deficits (measured in novel object recognition test), as well as with the preservation of pyramidal neurons of the mPFC and the CA1 layer of the hippocampus (measured with the marker of deep cortical layers Ctip2 and NeuN for the mature neurons, respectively), in comparison with those animals treated with vehicle. This neuroprotective effect was accompanied by a decrease in the inflammatory response, reflected in the decrease in the immunoreactivity for S100-β (a marker of mature reactive astrocytes) in the mPFC, and for Iba-1 (a marker of microglia) in the CA1 layer.

In summary, our data suggest the interest of elevating the endocannabinoid tone as a therapy against TDP-43-induced neuropathology in FTD, as it may serve to limit glial reactivity, to preserve neuronal integrity and to restore the cognitive domains.

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IVV-16: Investigating TDP-43 in the cerebellum of non-transgenic and TDP-43 mouse models throughout aging.

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Live Poster Session B, December 10, 2020, 5:10 PM - 5:50 PM

Background:
The most common pathological hallmark of ALS is the cytoplasmic mislocalisation and aggregation of ubiquitinated TDP-43, with the concurrent loss of functional nuclear TDP-43 in the motor neurons of the spinal cord and motor cortex. The cerebellum shares vast connectivity with these areas and functions in fine motor tuning yet seems spared from TDP-43 pathology, although inclusions of the ubiquitin-binding scaffold protein p62 have been identified in the cerebellar cortex of C9orf72 patients[1]. Despite the lack of TDP-43 pathology, its concentration within the cerebellum is three-fold higher than other brain regions[2]. Furthermore, differences in other neurodegenerative disease associated proteins have been seen between the cortex and cerebellum, with levels of mature APP and tau being lowest in the cerebellum[3],[4]. This suggests there may be an autonomous proteome and potentially different TDP-43 function or processing within the cerebellum.

Objectives:
To compare TDP-43 expression and cellular distribution throughout aging in the cerebellum and cortex of non-transgenic (NTg), hTDPWT and hTDPQ331K mouse models[5].

Methods:
Brains were harvested from 3 to 24-month-old NTg, hTDPWT and hTDPQ331K animals and processed for cortex and cerebellar specific cellular fractionation and western blotting, or immunohistochemistry. N and C-terminal derived TDP-43 antibodies (Proteintech) were used to detect TDP-43 levels in the various fractions and using brightfield (DAB) and fluorescence immunohistochemistry.

Results:
Western blot and immunohistochemical analysis of TDP-43 shows protein levels to be much higher in the cerebellum versus the cortex in control and transgenic mouse models. Cellular fractionation revealed a relative increase in cytoplasmic TDP-43 load in the cerebellum compared to the cortex in all mice, that is recapitulated with immunohistochemical staining. Studies comparing N and C terminal specific TDP-43 antibody staining have revealed antibody specific differential patterns of TDP-43 distribution between the cortex and cerebellum, suggesting that differently spliced, cleaved or folded TDP-43 may be present in the cerebellum compared to the cortex.

Conclusion:
The cerebellum is able to function with high concentrations of cytoplasmic TDP-43 whilst avoiding TDP-43 cytoplasmic aggregation. This suggests the cerebellum may be resistant to aberrant TDP-43 aggregation. Furthermore, differences in N and C-terminally stained TDP-43 in the cerebellum compared with the cortex suggest different isoforms of TDP-43 may exist within the cerebellum with potentially different functional abilities.

References:
1. King et al., Neuropathol. 2009; 29:466-471
2. Hu et al., Front. Aging Neurosci. 2017; 9:1.14
3. Causevica et al., Neurosci. Lett. 2010; 485:162-166
4. Gu et al., Nucleic Acids Res 2017; 45:6177-6193
5. Mitchell et al., Acta Neuropath Comms 2015; 3:36

IVV-17: Is reduced cytoplasmic dynein function a cause and a risk factor of MND?

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Live Poster Session B, December 10, 2020, 5:10 PM - 5:50 PM

Motor neurons (MNs) are highly polarised, with axons which can reach 1 m in length, and are consequently critically dependent on axonal transport for survival. There is increasing evidence that impaired intracellular trafficking is present in amyotrophic lateral sclerosis (ALS) and that it may contribute to the pathogenesis of the disease. Autosomal dominant point mutations in cytoplasmic dynein (dynein), the motor carrying cargoes in the retrograde direction, are associated with hereditary forms of motor neuron diseases. Yet whether reduced dynein function is sufficient or a risk factor for ALS remains elusive, likely due to the absence of appropriate genetic tools to manipulate dynein specifically in the MNs.

However, crossing a recently developed mouse model, in which exons encoding the ATP-binding domain of the dynein heavy chain (Dync1h1) have been flanked by two loxP sites, with a mouse strain expressing CRE under the ChAT promoter allows the ablation of dynein motor activity specifically in cholinergic neurons, including MNs. Therefore, we aimed to utilise this model in combination with the Legs at odd angles (Loa) model of dynein dysfunction (F580Y substitution on Dync1h1) to generate a spectrum of functional dynein to determine if impaired dynein in MNs is sufficient to trigger ALS-like pathology. Loa homozygous mice have a severe loss of MNs by E18.5 and die shortly after birth. Conversely, Loa heterozygous mice show a progressive lower limb weakness but a normal life span.

Combining these models, we have generated mice expressing ChAT+/Cre and one of four Dync1h1 genotypes in cholinergic neurons: wild type (Dync1h1 +/+), hemizygous wild type (Dync1h1 +/flx), Loa heterozygous (Dync1h1 +/loa) and Loa hemizygous (Dync1h1 loa/flx). Longitudinal characterisation over 52 weeks has shown a reduction in body weight in comparison to the wild type mice in the Loa heterozygous mice but more severely in the Loa hemizygous mice. The hemizygous wild type mice did not show any significant differences. Furthermore, male Loa hemizygous mice exhibit a steeper decline in muscle strength (Kondziela's inverted screen test) in comparison to Loa heterozygous mice between 4 and 16 weeks of age. Analysis of neuromuscular junctions (NMJs) in the hind limb lumbrical muscles also showed abnormalities, including a reduction in post synaptic acetylcholine receptor area and overall motor end plate area. These abnormalities may be indicative of developmental defects during NMJ formation. Importantly, the disassembly of NMJs and consequent muscle denervation is a critical factor in ALS pathogenesis. Based on our current data, we tentatively postulate that defects in dynein cause aberrant NMJ formation, potentially making them vulnerable to other ALS-associated risk factors and subsequent 'dying back' of the axons later in life. Further analysis will reveal whether other ALS-associated pathologies, including protein aggregation, are present in these mice.

IVV-18: Locomotor deficits in ALS mice are paralleled by loss of V1 interneuron connections onto fast motor neurons

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Live Poster Session B, December 10, 2020, 5:10 PM - 5:50 PM

ALS is characterized by the preferential degeneration of motor neurons innervating fast-twitch fatigable muscle fibers[1]. This work shows that fast fatigable motor neurons receive 2.8 folds more glycinergic inputs than the slow-twitch fatigable resistant ones, which are more resistant to the disease[2]. Quantifications of soma-near synapses in a SOD1G93AGlyT2GFP mouse model, revealed 50% loss of glycinergic synapses restricted to fast fatigable motor neurons by postnatal day 45, a time point in which both neuromuscular junctions and motor neurons are still spared. When focusing on the V1 Engrailed-1 positive inhibitory interneurons, that control the speed of locomotion in mammals and are known to express glycine[3], we found the same connectivity pattern in physiological conditions and disease. Importantly, at postnatal day 63, SOD1G93A mice had a reduction to 13% of the V1 soma-near synapses2. The connectivity loss observed between postnatal day 45 and 63 was paralleled by the development of locomotor deficits - assessed by DeepLabCut tracking and post-tracking analysis[4] – and was seen as loss of speed and decrease in step frequency and stride length. The appearing of this locomotor phenotype was defined as ‘Onset of locomotor phenotype’. To elucidate if the synaptic loss onto fast fatigable motor neurons could explain the changes in the locomotor phenotype, we performed selective silencing of V1 interneurons in the spinal cord utilizing inhibitory DREADDs[5]. An intersectional mouse model was used to allow for dual-recombinase and iDREADDs expression in spinal V1 interneurons. Importantly, the reversible dampening of V1 interneurons phenocopied the ALS-induced reduction in speed in the control mice but did not have any effects in the SOD1G93A animals after the appearing of ‘Onset of locomotor phenotype’, demonstrating that the iDREADDs effect was occluded in the ALS mice. Overall, this study shows that spinal vulnerable and resistant motor neurons receive different amount of inhibitory inputs, which are selectively lost only on the vulnerable ones during early stages of the disease, leading to locomotor deficits. Strikingly, loss of connectivity with V1 interneurons leads to reduction of speed and stride length, symptoms which were previously observed in ALS patients. Future directions are now focused on finding early diagnosis markers specific for interneuron-motor neuron connectivity loss, as well as rescuing the synaptic connectivity between V1 interneurons and fast fatigable motor neurons, in order to investigate if such treatment could reduce ALS-induced excitotoxic events and motor neuron degeneration.

1) Pun S et al (2006) Nat Neurosci 9(3):408-19
2) Allodi I et al https://doi.org/10.1101/2020.06.23.166389
3) Gosgnach S et al (2006) Nature 440:215-19
4) Mathis A et al (2018) Nat Neurosci 21:1281-89
5) Ray RS et al (2011) Science 333:637-42

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IVV-19: Loss of cyclophilin A function promotes TDP-43 proteinopathy: implications for ALS and FTD.

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Live Poster Session B, December 10, 2020, 5:10 PM - 5:50 PM

Background:
Cyclophilin A (PPIA) is a multifunctional protein that has been associated with different human diseases, but its role in pathogenesis is still unknown. We first associated PPIA with nervous system degeneration, identifying it as a translational biomarker of ALS, with a protective role in ALS pathology [1-2]. We found that PPIA levels in peripheral blood mononuclear cells (PBMCs) could distinguish ALS patients from controls, suggesting a diagnostic potential [1-3]. We observed that absence of PPIA in an animal model of ALS exacerbated disease progression and low level of PPIA correlated with a worse disease phenotype in ALS patients, suggesting a prognostic potential [2-4]. We demonstrated that PPIA, once lysine acetylated (acetyl-PPIA), interacts with TDP-43 and regulates some of its functions [2]. Interestingly, we detected low acetyl-PPIA in PBMCs of sporadic ALS patients [2].

Objective:
TDP-43 is a key player in ALS pathogenesis. TDP-43 cytoplasmic mislocalization, fragmentation, hyperphosphorylation and aggregation are pathological hallmark in ALS and FTD patients. The molecular mechanisms at the basis of TDP-43 pathology have not been elucidated yet. Here we investigated PPIA function as a player of this process.

Methods:
We characterized PPIA knock-out mice (PPIAko) throughout their entire lifespan and performed MRI analysis, histology, electrophysiology, cognitive and motor tests, evaluation of neuroinflammation and TDP-43 pathology. We screened ALS patients for coding, non-synonymous and loss-of-function SNPs in the PPIA gene. We performed molecular dynamics (MD) simulations of PPIA structures. We studied function of PPIA mutants in vitro.

Results:
PPIAko mice develop an FTD-like phenotype with marked TDP-43 pathology. We identified a sporadic ALS patient carrying a mutation in the PPIA gene that results in a substitution of a lysine residue for a glutamate residue, and negative to other ALS and hereditary paraparesis gene mutations. MD analyses revealed a prominent structural variation between wild-type and mutant PPIA. In vitro we observed that PPIA mutation accelerates PPIA degradation and impairs its interaction with TDP-43.

Discussion and conclusions:
PPIA is worthy of further investigation since its absence in mice promotes a neurodegenerative disease with key features of FTD, and a loss-of-function mutation in PPIA gene was found in an ALS patient. We confirmed the importance of PPIA for the stability and function of TDP-43.

References:
[1] Nardo G, Pozzi S, Pignataro M et al PLoS One 2011; 6:e25545; [2] Lauranzano E, Pozzi S, Pasetto L et al Brain 2015; 138(Pt 4):974-91; [3] Filareti M, Luotti S, Pasetto L et al Front Mol Neurosci 2017; 6;10:99; [4] Luotti S, Pasetto L, Porcu L et al Neurobiol Dis 2020; 139:104815

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IVV-20: Neuroprotective effects of Sigma 1 receptor ligands on motoneuron death induced by spinal root injury

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Live Poster Session B, December 10, 2020, 5:10 PM - 5:50 PM

Background:
Spinal root injury represents a proximal axotomy that results in fast progressive death of motoneurons (MNs) in the ventral horn and a neuroinflammatory response.

Objectives:
We have assessed the evolution of MN degeneration caused by L4-L5 root injury in adult mice. We also evaluated if administration of the Sigma-1 receptor ligand, PRE-084 promotes MN survival after the injury, and which mechanisms may be involved in the neuroprotective effects.

Methods:
Following partial laminectomy, the L4 and L5 spinal roots were cut at the postganglionic level. The lumbar spinal cord was studied at 7, 14, 28 and 42 days post-injury by means of histological, immunohistochemical and western blot techniques.

Results:
In the ventral horn of the injured side there was a loss of 30% and 40% of spinal MNs at 28 and 42 days, respectively, compared with uninjured mice. Microglia and astroglia reactivity were markedly increased in the injured cord segments at 28 days, and slightly reduced at 42 days post-injury. PRE-084 treatment increased by 20% the number of surviving MNs and significantly reduced glial reactivity after rhizotomy. Molecular analyses showed a modulation of endoplasmic reticulum stress and autophagic markers after the injury.

Conclusions:
These findings evidence that the Sigma-1 receptor is a potential target for the treatment of MN degeneration.

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IVV-21: P2X7 activation enhances skeletal muscle metabolism and regeneration in SOD1G93A mouse model of Amyotrophic Lateral Sclerosis

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Live Poster Session B, December 10, 2020, 5:10 PM - 5:50 PM

Muscle weakness plays an important role in neuromuscular disorders comprising amyotrophic lateral sclerosis (ALS). However, it is not established whether muscle denervation originates from the motor neurons, the muscles or more likely both. Previous studies have shown that the expression of the SOD1G93A mutation in skeletal muscles causes denervation of the neuromuscular junctions, inability to regenerate and consequent atrophy, all clear symptoms of ALS. In this work, we used SOD1G93A mice, a model that best mimics some pathological features of both familial and sporadic ALS, and we investigated some biological effects induced by the activation of the P2X7 receptor in the skeletal muscles. The P2X7, belonging to the ionotropic family of purinergic receptors for extracellular ATP, is abundantly expressed in the healthy skeletal muscles, where it controls cell duplication, differentiation, regeneration or death. In particular, we evaluated whether an in vivo treatment in SOD1G93A mice with the P2X7 specific agonist 2’(3’)-O-(4-Benzoylbenzoyl) adenosine5’-triphosphate (BzATP) just before the onset of a pathological neuromuscular phenotype, could exert beneficial effects in the skeletal muscles.

Our findings indicate that stimulation of P2X7 improves the innervation and metabolism of myofibers, moreover elicits the proliferation/differentiation of satellite cells, thus preventing the denervation atrophy of skeletal muscles in SOD1G93A mice. Overall, this study suggests that a P2X7-targeted and site-specific modulation might be a strategy to interfere with the complex multifactorial and multisystem nature of ALS.

IVV-22: Peripheral administration of SOD1-containing spinal cord homogenates do not transmit fatal ALS in transgenic mice

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Live Poster Session B, December 10, 2020, 5:10 PM - 5:50 PM

Background:
The deposition of aggregated proteins is a common neuropathological denominator for common age-related neurodegenerative disorders. Experimental evidence suggests that disease propagation involves prion-like mechanisms that cause the spreading of template-directed aggregation of disease-associated proteins. In transgenic (Tg) mouse models of SOD1-provoked ALS, minute amounts of aggregate seeds inoculated into the spinal cord or peripheral nerves induce template-directed SOD1 aggregation that spreads along the neuroaxis and causes fatal ALS-like disease. This infectious nature of spreading pathology may have implications for the safety of caregivers and medical personnel, for surgical procedures, and recipients of donated blood or tissue.

Objectives:
To investigate whether the efficient spread of SOD1 aggregation and ALS-like disease is unique to spinal cord and peripheral nerve inoculations or if SOD1 aggregates have a potential to spread from the periphery into the CNS.

Methods:
We inoculated human SOD1 (hSOD1) aggregate seeds into the peritoneal cavity or hind-limb skeletal muscle of asymptomatic Tg mice expressing mutant hSOD1 and compared to ALS-like phenotype induced by spinal cord inoculations.

Results:
Our results suggest that peripheral administration of large amounts of hSOD1 aggregate seeds does not transmit seeded aggregation to the CNS or premature ALS-like disease in hSOD1 Tg mice. Nor was any hSOD1 aggregation detected in the liver, kidney, skeletal muscle, or peripheral nerve. To explore potential reasons for the lack of disease transmission, we examined the stability of hSOD1 aggregates and found them to be highly vulnerable to both detergent and proteases.

Discussion:
Our findings suggest a low risk of transmission for potentially exposed individuals and medical personnel handling ALS patient samples. Furthermore, we found that mutant SOD1 aggregates can easily be degraded by detergent and proteases, which limits the potential for transmission.

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IVV-23: The response to whole body hyperthermia is defective in a TDP-43 M337V mouse model

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Live Poster Session B, December 10, 2020, 5:10 PM - 5:50 PM

Given that 90% of ALS cases are without obvious genetic etiology (sporadic), environmental components are suspected as a contributing factor in ALS pathogenesis. Development of effective therapies requires a deeper understanding of how the environment impacts the cell biology governing disease initiation and progression. Misprocessing of stress granules has been associated with acceleration of disease course in fly and mouse models of FUS-mediated ALS subjected to traumatic brain injury and arsenite as external stressors[1 2]. Similarly, thermal stress has been used to induce stress granule formation in a mouse tauopathy model[3]. Two hypotheses exist for how stress granule dysfunction is associated with ALS pathogenesis: 1) that stress granules could be the basis of the presumably toxic cytoplasmic inclusions observed in patient neurons or 2) that a compromised stress response causes improper stress granule formation and increases vulnerability in motor neurons. In either case, defective stress granule dynamics would ultimately lead to toxicity and premature cell death.

In this study, we use TDP-43 M337V mice which feature a single copy insertion of the human mutant TDP-43 allele, display motor phenotypes, but do not exhibit TDP-43 pathology in neurons[4]. Therefore, applying an external stress allows us to test both hypotheses regarding stress granule formation in ALS. We subjected TDP-43 M337V mice and negative littermates to hyperthermic shock at different ages and examined stress granule dynamics in neurons. We find that in wild type mice subjected to hyperthermic stress, TIAR-positive granules and redistribution of polyadenylated mRNA are detected in vivo, but are not evident in age-matched TDP-43 M337V mice. Interestingly, granule formation proceeds normally in SOD1 G93A mice indicating that the defect is unique to the TDP-43 M337V model. We suggest that the observed defect in granule assembly correlates with stoichiometric alterations of key stress granule proteins which may influence liquid-liquid phase separation properties in neurons. In addition, TDP-43 inclusions were not detected in spinal motor neurons. Our data are consistent with the hypothesis that motor defects could be associated with the improper formation of stress granules in this model.

References:

1. Anderson EN et al. (2018) Hum Mol Genet 27:1366–1381.
2. Zhang X et al. (2020) Brain 143:1350–1367.
3. Shelkovnikova TA et al. (2017) Cell Death Dis 8:e2788.
4. Gordon D et al. (2019) Neurobiology of Disease 121:148–162

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IVV-24: What is all the FUS about zebrafish?

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Live Poster Session B, December 10, 2020, 5:10 PM - 5:50 PM

Amyotrophic Lateral Sclerosis (ALS) is characterized by the relentless loss of motor neurons in the brain and spinal cord for which there is no cure or effective treatment to slow or halt the disease progression. Missense mutations in the gene Fused in Sarcoma (FUS) are associated with dominantly-inherited familial ALS cases. FUS is an RNA binding protein that belongs to the FET (FUS, EWS and TAF15) family of RNA binding proteins which have roles in transcription, splicing, RNA transport, translation control and RNA degradation that are modulated by highly conserved domains. About a third of the ALS-causing missense mutations in FUS are localized to exon 15, encoding the Nuclear Localization Signal (NLS).
Antisense oligonucleotides (ASOs) are modified single-stranded oligodeoxynucleotides that can reduce expression of target mRNA and have shown success as therapies in targeting genetic disorders. A new clinical research program plans to explore the use of an experimental ASO in 8 ALS-FUS patients. These treatment plans are coined “N-of-1” therapies and open a door into the FDA acceptance of potential therapies with very small sample sizes. It is very important to first evaluate ASOs in a recapitulating animal model, as it may help to identify the best target sequence and shed light on any adverse effects in response to a FUS-ASO. Through the use of the CRISPR/Cas9 system, we have generated the analogous FUSR521H variant (fusR536H knockin (KI) mutation in a Danio rerio (Zebrafish) model as well as a zebrafish knockout model (fus -/-)). We present preliminary data characterizing the degenerative motor phenotype in our homozygous R536H variant zebrafish and compare motor phenotypes, protein expression, autoregulation deficits, immunofluorescence analysis and RNA sequencing analyses within fus+/+, fusR536H/R536H and fus-/- zebrafish lines. These preliminary results indicate a clear degenerative phenotype in our R536H variants that is distinct from the behaviour, expression and analysis seen in our wildtype and knockout lines leaving much promise for this model to be used in testing different experimental ASO therapies.

IVV-25: Evaluation of M102 as a Novel Therapeutic in Amyotrophic Lateral Sclerosis (ALS)

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Live Poster Session C, December 11, 2020, 12:05 PM - 12:50 PM

Background:
ALS is a devastating neurodegenerative disease for which new therapies are urgently needed. We have identified M102, a small molecule new chemical entity (NCE) which activates the NFE2-related factor 2-antioxidant response element (NRF2-ARE) pathway, as a promising drug candidate for the treatment of ALS. More recently, we have identified that M102 can also activate the heat shock factor 1 (HSF1) signalling pathway, activating transcription of heat shock element (HSE) associated genes that upregulate molecular chaperones to improve proteotoxic stress. Importantly, we have shown that activation of the NRF2 and HSF1 pathways occurred at comparable M102 doses. Here we extend our previous findings on M102 and investigate its pharmacology and efficacy in a TDP-43Q331K mouse model, which is more relevant to sporadic ALS.

Objectives:
To investigate the pharmacology and efficacy of M102 in a TDP-43Q331K mouse model of ALS.

Methods:
Two cohorts of TDP-43Q331K transgenic mice were dosed subcutaneously with vehicle or M102 (2.5mg/kg twice daily, or 5mg/kg once daily) from 25 days of age. One cohort was dosed until 6 months (n=14/group), while the other was dosed until 3 months (n=6/group). Behavioural tests for motor function (rotarod, gait analysis), cognitive function (marble burying), and muscle function (electrophysiology) were measured at various time points during the study. Body weights were also recorded daily. At the end of the study, brain and spinal cord were collected for immunohistochemistry and qPCR analysis.

Results:
In the 6-month cohort, a significant decrease in body weight was observed for the 2.5mg/kg M102 dosing group, compared to the vehicle group. In addition, behavioural analysis showed improvements in compound muscle action potential (CMAP) and gait parameters in both M102 dosing groups at 6 months of age, compared to the vehicle group. Tissue analysis showed significant upregulations in NRF2 and HSF1 target genes in the cortex at both 3 and 6 months of age. Among different dosing groups, staining of lumbar spinal cords exhibited no statistical significant difference in astrocyte or microglia markers.

Discussion:
In the TDP-43Q331K mouse model, M102 activated both NRF2 and HSF1 pathways, and significantly improved motor functions, when dosed at 2.5 mg/kg twice daily or 5 mg/kg once daily. Based on data from this and previous studies, we conclude that M102 is a promising disease modifying drug candidate for the treatment of ALS. Additional effort is undertaken for the development of M102 in preclinical and clinical studies.

IVV-26: Flow cytometric analysis of dissociated cells can be used to detect disease phenotypes and screen drug candidates in zebrafish models of ALS

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Live Poster Session C, December 11, 2020, 12:05 PM - 12:50 PM

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterised by progressive loss of motor neurons in the central nervous system. Human ALS disease phenotypes can be modelled in zebrafish (Danio rerio) via expression of mutated ALS-causing genes. Expression of ALS-causing genes in transgenic zebrafish can produce impaired movement, motor neuron abnormalities and formation of insoluble protein aggregates, akin to disease phenotypes found in ALS patients. Due to these similarities with the human disease, zebrafish models can be used for high throughput screening of novel therapeutics.

In our lab, we examine swimming behaviour in 2-day post-fertilisation zebrafish larvae as a readout of efficacy of therapeutic agents. More recently, we have developed novel flow cytometry assays that can be used as additional readouts of therapeutic efficacy following the assessment of swimming behaviour. We have modified a previously reported method of flow cytometric analysis of inclusions and trafficking (FloIT) to investigate detergent-insoluble protein aggregates in vivo. FloIT analysis has revealed the presence of detergent-insoluble protein aggregates in transgenic zebrafish expressing mutant (R521C) human Fused in Sarcoma (FUS)-EGFP. Further, we have found that 24-hour treatment of FUS-EGFP larvae with compounds that modulate the autophagy protein quality control pathway can alter the number of insoluble aggregates detected by FloIT. Thus, our in vivo FloIT approach enables identification of novel treatments that aid autophagic degradation aggregated proteins. Further, dissociated cells can be stained with fluorescent markers of oxidative stress and cell death. We have found that novel compounds that are found to rescue impaired swimming in zebrafish larvae transiently expressing mutant (S216G) human Cyclin-F (CCNF) (with mCherry fusion protein) also decrease the number of ROS+ and necrotic cells, protecting against oxidative stress and cell death.

In conclusion, flow cytometric analysis of dissociated zebrafish cells, for example to allow quantification of the number of protein aggregates or stressed and necrotic cells, can be used to compliment behavioural testing within preliminary drug screens, providing additional evidence of treatment efficacy.

IVV-27: Investigating brain glucose and amino acid metabolism in a TDP43Q331K mouse model of Motor Neurone Disease

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Live Poster Session C, December 11, 2020, 12:05 PM - 12:50 PM

Background:
Impairments in energy metabolism have been hypothesized to contribute to the onset and progression of Motor Neurone Disease (MND) [1]. We have previously described extensive alternations in brain glucose and amino acid metabolism in the SOD1G93A mouse model of MND [2-3]. Whether these alterations are broadly exhibited, and therefore disease-relevant across multiple models of MND remains unknown.

Objective:
Characterise brain glucose and amino acid neurotransmitter metabolism in the TDP43Q331K mouse model of MND.

Methods:
To investigate glucose and acetate metabolism, we injected labelled 543 mg/kg [1-13C] glucose and 504 mg/kg [1,2-13C] acetate i.p. to female wild-type (WT) and TDP43Q331K mice (n=10-12) at 90 days of age (early symptomatic). Fifteen minutes following injection, mice were sacrificed, and the cerebral cortex was immediately collected. Metabolites were extracted with water, methanol and chloroform. We measured total as well as labelled glucose and acetate derived metabolite levels using 1H and 13C nuclear magnetic resonance spectroscopy. Total amounts of amino acids were measured with high-pressure liquid chromatography (HPLC).

Results:
We found a 45% and 18% reduction in levels of taurine ($p<0.0001$) and N-acetylaspartate ($p<0.01$) in TDP43Q331K cortex respectively when compared to WT mice. By contrast, levels of L-serine ($p<0.05$) and phosphocholine ($p<0.0001$) were increased in TDP43Q331K mice by 22% and 38% respectively. The total amounts of alanine were increased in TDP43Q331K cortex without changes in [1-13C] glucose derived [3-13C] alanine and [3-13C] lactate as well as total lactate. The levels of the first turn TCA cycle metabolites [4-13C] glutamate, [4-13C] glutamine and [2-13C] GABA were comparable between WT and TDP43Q331K mice. No changes were observed in [1,2-13C] acetate derived metabolites such as [4,5-13C] glutamate, [4,5-13C] glutamine and [1,2-13C] GABA.

Conclusion:
Our data suggest that despite normal glycolysis, entry of pyruvate into the TCA cycle, and acetate metabolism, TDP43Q331K mice exhibit early impairments in amino acid metabolism in the cortex. The altered levels of amino acids and metabolites in TDP43Q331K mice may be indicative of oxidative damage, mitochondrial dysfunction, compensatory responses to reduce ER stress, and abnormalities in lipid turnover or cholinergic transmission. Some of the metabolic changes observed in TDP43Q331K mice were distinct from those of SOD1G93A mice.

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References:
[1] L Dupuis et al., Proc Natl Acad Sci U S A 101 (30), 11159 (2004); [2] TW Tefera and K Borges, J Cereb Blood Flow Metab 39 (9), 1710 (2019); [3] TW Tefera et al., Mol Neurobiol 56 (8), 5844 (2019).

IVV-28: New model approaches for understanding axon degeneration in ALS

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Live Poster Session C, December 11, 2020, 12:05 PM - 12:50 PM

Axon degeneration is a key feature of amyotrophic lateral sclerosis (ALS), however the mechanisms which drive these cellular changes are unclear. Currently, appropriate models which recapitulate axon degeneration are lacking. We have developed a novel visual system model which allows introduction of genetically altered proteins, such as those observed in disease, or drugs to mimic disease processes. These 'insults' affect the retinal ganglion cells (RGCs) in the retina, which have similar features to other CNS neurons implicated in ALS, and allows for detailed analysis of the cell bodies, axons and synapses of these cells. In our lab, we have been examining two key features of ALS and their effects on axon degeneration.
The first pathologic process investigated was mislocalisation of TDP-43, which occurs in 90% of ALS cases. TDP-43 with a defective nuclear localisation sequence (TDP-NLS) or wildtype TDP-43 (TDP-WT) were packaged into adeno-associated virus 2 (AAV2) and, along with PBS vehicle control, were delivered intravitreally to C57Bl6 adult mice (n=5/treatment). After 3 months, tissue was harvested and analysis demonstrated that TDP-WT resulted in synaptic alterations in the retina ($p<0.05$), while TDP-NLS resulted in neurofilament alterations in the retina ($p<0.05$) and optic nerve($p<0.05$). Overexpression of TDP-43 in general (TDP-WT and TDP-NLS) resulted in decreased numbers of synapses in the optic targets of the brain.
Excitotoxicity, a pathologic process known to occur in ALS, was also examined in a separate cohort of mice. The excitotoxin, kainic acid (KA), or PBS vehicle were administered intravitreally to C57Bl6 mice (n=10/treatment). The optomotor response was used to measure visual acuity prior to injection, and at 1- and 7-days post-injection. 1nM KA resulted in a loss of visual acuity 1 day after injection which was sustained at the 7-day timepoint. In contrast, eyes treated with >1nM KA failed to exhibit a response at any point after injection ($p<0.001$), while the performance of eyes injected with PBS was not significantly affected. Tissue analysis 7 days after treatment revealed significant axonal loss ($p<0.05$) and alterations to axonal structure following KA treatment. We are currently using this model to determine therapeutic targets including microtubule stabilisation, and removal of a key mediator of Wallerian degeneration, SARM1.
This novel visual system model is a positive step forward for recapitulating disease processes in an in vivo setting, and allows for rapid screening of therapeutic compounds and examination of the effects at the cellular level.

IVV-29: Pharmacological blockade of metabotrobic glutamate receptor 5 by the negative allosteric modulator CTEP improves the disease course of ALS in SOD1 G93A mice

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Live Poster Session C, December 11, 2020, 12:05 PM - 12:50 PM

Background:
The pathogenesis of ALS is not fully clarified, although excessive glutamate (Glu) transmission and the downstream cytotoxic cascades are major mechanisms for motor neuron death. Metabotropic Glu receptor 1 and 5 (mGluR1,5) may represent potential therapeutic targets, as they are over-expressed in ALS (1, 2, 3, 4) and regulate cellular processes modified during disease progression (3, 5, 6). We previously reported that mGluR5 expression and function are already altered at early symptomatic disease stages in the SOD1G93A mouse model of ALS (7) and that knocking-down mGluR1 or mGluR5 in SOD1G93A mice improved the disease scenario (8, 9). In particular, the genetic ablation of mGluR5 delayed the pathology onset and improved survival probability. These effects were associated to ameliorated motor skills, preserved MNs, decreased astrocyte and microglia activation, reduced cytosolic Ca2+ (10).

Objectives:
Building on the genetically-induced functional and biochemical background of mGluR5-deficient SOD1G93A double mutant mice, we here explored the effects of the chronic pharmacological treatment of SOD1G93A mice with 2-chloro-4-((2,5- dimetthyl-1-(4-(trifluoromethoxy)phenyl)-1H-imidazol-4-yl(ethynyl)pyridine (CTEP), an orally available negative allosteric modulator of mGluR5.

Methods:
We treated SOD1 G93A mice with CTEP at the doses of 2 mg/kg/48h or 4 mg/kg/24h starting from 90 day of life, an early symptomatic disease stage. Disease progression was studied by behavioural and histological approaches.

Results:
CTEP, dose dependently ameliorated clinical features in SOD1 G93A mice. The low CTEP dosage increased survival and improved motor skills in female mice only, since it only barely produced positive effects in male mice. The high dosage significantly ameliorated disease symptoms and survival in both males and females, being females always more responsive to the drug treatment. CTEP treatment also reduced motor neurons death, astrocyte and microglia activation and abnormal Glu release in the spinal cord of treated mice.
Conclusions:
Our previous genetically-based work and the present pharmacological results suggest that mGluR5 represents a promising target for the treatment of ALS and highlights the inhibitors of mGluR5 function as favourable new pharmacological tools with a possible translational perspective.

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References:
1 Anneser et al., 1999; Neurobiol Dis. 6(2):140-147.
2 Aronica et al., 2001; Neuroscience, 105:509-520.
3 Valerio et al., 2002; Pharmacol. Biochem. Behav. 73 (2), 447-454.
4 Brownell et al., 2015; J Neuroinflam,12:217.
5 Rossi et al., 2008; Cell Death Diff., 15: 1691–1700.
6 D'Antoni et al., 2008, Res. 33 (12): 2436–2443
7 Bonifacino et al., 2019, Neurobiol Dis.,129:79-92.
8 Milanese et al., 2014; Neurobiol. Dis, 64: 48–59.
9 Bonifacino et al., 2017; Neuropharmacology, 123, 433–445.
10 Bonifacino et al., 2019¸ Int. J. Mol. Sci., 20(18):4552.

IVV-30: Physiological models of TDP-43 ALS including a new fully humanised Knock In model

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Live Poster Session C, December 11, 2020, 12:05 PM - 12:50 PM

Mouse models are critical to further our understanding of disease processes. To try to recapitulate as faithfully as possible human ALS disease pathogenesis, we have a long-term interest in creating knock in (KI) mouse models of ALS. Our methods of creating KI mice range from chemical mutagenesis through to CRISPR/Cas9 and sophisticated targeting constructs to replace entire mouse loci with the human genomic orthologues including introns and exons. Here we present data for three different TDP-43 KI mouse models:

1. M323K (LCDmut): This strain carries a point mutation in TDP-43 (M323K). Homozygous mutants develop progressive motor abnormalities underlined by motor neuron degeneration. Here we have expanded on our published behavioural assessment1, to better assess the onset of motor abnormalities as well as identify new cognitive deficits.
2. Q331K: This strain carries a known pathogenic KI point mutation in TDP43 (Q331K). Here we include behavioural analysis of this strain on a B6 and B6/DBA background as well as cellular and molecular analysis of derived primary cell lines.
3. Humanised KI model: We present a new mouse on which we have replaced the complete mouse Tardbp gene with human TARDBP, from the start to the stop codon, including all introns in between, producing the first mouse model expressing a human TDP43 protein physiological expression from the endogenous locus. Here we include molecular evidence of the genomic engineering from humanised mice.

All these TDP-43 mice are enabling us to particularly focus on identifying early stage disease processes in more physiological mammalian models.

IVV-31: The correlative presence of misfolded SOD1, mitochondrial vacuolization and microgliosis defines distinct phenotypes of motor neuron damage during the progression of disease in the SOD1G93A ALS mice

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Live Poster Session C, December 11, 2020, 12:05 PM - 12:50 PM

Background:
Accumulation of misfolded SOD1 is a hallmark of motor neuron (MN) pathology in familial ALS. Mitochondrial vacuolization, microgliosis and MN loss are also central elements in ALS. There is also an important variability in the vulnerability of distinct MN subtypes in ALS, which appears to be correlated with different characteristics of their excitability. C-boutons are cholinergic inputs to α-MNs which play an important role in the regulation of MN excitability. However, the contribution of C-boutons to ALS pathology is controversial.

Objectives:
In an attempt to rationalize the relationship between the mentioned pathological aspects which converge in MN degeneration, we performed a quantitative correlative analysis of the neuroinflammatory reaction, misfolded SOD1 (mfSOD1) accumulation and C-bouton alteration in the SOD1G93A ALS mice. The effects of a supplementary stress on ALS MNs induced by peripheral nerve injury were also assessed.

Methods:
SOD1G93A mice were used. Sciatic nerve axotomy were performed according to stablished procedures. Multiple fluorescent immunolabeling was performed on spinal cord cryostat sections and observed under the confocal microscope. Some tissue samples were also processed for electron microscopy.

Results:
The immunocytochemical analysis with the C4F6 antibody allowed us to define three MN phenotypes. In phenotype 1, there was no evidence of mfSOD1 accumulation, but microglia started to contact the surface of MNs and the postsynaptic organization of C-boutons was significantly impaired; however, the C-bouton presynaptic compartment remained unaltered. In phenotype 2, there was a significant accumulation of mfSOD1 in MN neuropile together with important microgliosis; however, no changes in C-boutons were observed. In phenotype 3, there was a significant increase in mfSOD1 accumulation in both the neuropile and MN somata; microglial cells were still active and markers of both pre- and post-synaptic C-bouton compartments were significantly reduced. After sciatic nerve axotomy performed in a presymptomatic stage, MNs worsen their phenotype, emulating that predominating in later stages of disease in non-axotomized animals. However, when axotomy was performed in early symptomatic stages, the added stress provoked additional microgliosis, but this did not result in an intensification of the proportion of worse MN phenotypes.

Discussion and conclusions:
By classifying MNs according to the amount mfSOD1 aggregates, we can dissociate the severity of alteration in individual MNs from the time-course points of disease. Compensatory mechanisms acting in early disease stages contribute to hide, in clinical terms, the severe pathological changes that appear early in the most vulnerable MN population. Any added stress can substantially worsen the MN pathology phenotype in early but not in late stages of the disease.

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IVV-32: Updates on seeding studies: SOD1 prions transmit aggregation and fatal ALS-like disease – Introducing Strain C

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Live Poster Session C, December 11, 2020, 12:05 PM - 12:50 PM

Background:
In the last decade, evolving evidence suggest the presence of propagating prion-like species in several types of neurodegenerative diseases associated with misfolding of host-encoded non-mutated proteins. Among these are AD, PD, the tauopathies, HD and ALS. A hallmark of prions is the presence of different conformational aggregate strains with different biological activities. Another characteristic of prions is that subsequent prion passage within the same host can lead to a shorter incubation period. In ALS, using binary epitope mapping (BEM) we have identified two structurally different strains of mutant hSOD1 aggregates (named A and B) in the CNS of Tg mice models expressing full-length hSOD1 variants (1). When seeded into spinal cords of adult hSOD1G85R Tg mice, these strains transmit exponentially propagating templated hSOD1 aggregation selectively in the motor system with concomitant development of muscle wasting and paresis (2, 3).

Objective:
To further investigate prion-like properties of hSOD1 strains in ALS, and explore the spreading characteristic of the disease in vivo.

Methods:
Stereotaxic inoculation of hSOD1 aggregate strains into spinal cords of pre-symptomatic Tg-hSOD1D90A mice were performed. This mutation is essentially wt-SOD1 like (4). Normally, Tg-hSODD90A only develop motor neuron disease phenotype when the transgene is homozygous. Mice hemizygous for the hSOD1D90A transgene insertion do not spontaneously develop ALS pathology and have a normal murine lifespan (>700 days). First & second passage studies were performed to investigate further prion-like properties of hSOD1.

Results:
Inoculations of either strain A or B seeds into the lumbar spinal cord of 100-day-old hemizygous hSOD1D90A mice induced progressive hSOD1 aggregations and premature fatal ALS-like disease after ≈250 and ≈350 days, respectively. BEM analysis of aggregates in the terminal stage lead to a surprise discovery: Inoculation of strain A into hemizygous hSOD1D90A mice gave rise to a new strain named C, which has the C-terminal end apparently recruited to the aggregate core. In contrast, hemizygous hSOD1D90A mice inoculated with a mouse control seed died from senescence-related causes at ages >700 days.

Second passage inoculations were then performed in hemizygous hSOD1D90A mice, using spinal cord homogenates with strain C aggregates as seeds. The novel prion strain was much more efficient, and transmitted progressive hSOD1 aggregation and ALS-like disease which was fatal already 100 days after inoculation. The resulting conformational changes are under investigation.

Conclusion:
We provide further evidence of the similarities between hSOD1 and the prion protein. Our data suggest that mutations in SOD1 are inducing aggregation and ALS pathology via a prion mechanism.

References:
1. Bergh et al. PNAS. 2015
2. Ekhtiari Bidhendi et al. J Clin Invest. 2016
3. Ekhtiari Bidhendi et al. Acta Neuropathol. 2018
4. Andersen et al. Nat Gen. 1995

IVV-33: Utilising molecular imaging to investigate protein aggregate formation in vivo

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Live Poster Session C, December 11, 2020, 12:05 PM - 12:50 PM

Background:
Although ALS clinically manifest as a heterogeneous disease with varying onset and progression, a unifying feature is the presence of cytoplasmic TDP-43 aggregates. One aspect that remains unclear is the dynamic process of how these aggregates assemble and how they can form pathological inclusions. Clinical verification of aggregation pathology is mostly limited to histological techniques that provide a static snapshot of the aggregation pattern at predetermined stages. This significantly limits the ability to investigate the dynamic molecular mechanisms that are believed to trigger aggregate formation, maturation and mislocalization into the cytoplasm.

Objectives:
In this study we validated and optimized a fluorescence complementation approach to study protein-protein interactions of TDP-43 and Fus in vivo. We demonstrate the formation and localization of ALS-linked aggregates by combining Biomolecular Fluorescence Complementation (BiFC) with the advantages of zebrafish as a vertebrate model.

Methods:
Bimolecular fluorescence complementation (BiFC) is based on the reassembly of the unfolded, complementary, non-fluorescent N- and C-terminal fragments of a split fluorophore, which are fused to the protein of interest. Upon interaction of the protein of interest, the split fluorophore fragments are brought into spatial proximity (generally <7 nm), enabling the structural complementation of the fluorophore (and therefore fluorescence reconstitution). We used this technique to study aggregation formation of full-length human wild-type TDP-43 and zebrafish Fus (mRNA).

Results:
Here, we combined the power of BiFC with the advantages of the zebrafish system to validate and visualize the formation of ALS-linked aggregates in real time in a vertebrate model. We further optimized fluorescence complementation by introducing the mVenus I152L substitution into our constructs. We report preliminary findings on the dynamic aggregation of the ALS-linked hallmark proteins Fus and TDP-43 in their corresponding nuclear and cytoplasmic compartments using BiFC. Our in vivo analysis of TDP-43 compartmentalization confirmed an increased mislocalization of the mutant form of TDP-43 (M337V). Interestingly, co-expression of wild-type and mutant Fus BiFC fusion proteins resulted in fluorescence complementation in intra-nuclear aggregates, not cytoplasmic aggregates. Taken together, our results confirm that BiFC can be utilized to study ALS-linked aggregate formation and conceivably spread in vivo.

Discussion:
Combining the power of molecular imaging with the advantages of the zebrafish system, we present the first BiFC study assessing ALS aggregate formation in vivo. To the best of our knowledge, this BiFC approach may be one of the very few ways to assess how recruitment of mutant or pathological forms of TDP-43 can directly influence the wild-type TDP-43 (or vice versa). Identification of the mechanistic and molecular underpinnings of these aggregation processes has important implications for ALS and a range of other human proteinopathies.

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IVV-34: Validation of Stress Granule Reporters in Zebrafish.

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Background:
Currently, the pathological biochemistry of Amyotrophic Lateral Sclerosis (ALS) is poorly understood resulting in limited treatment options. Emerging research has implicated aberrant stress granules in ALS pathology (1). Stress granules are accumulations of non-membrane bound RNA-protein assemblies which aggregate in the cytosol of cells as a natural response to external stimuli (2). These stress granule assemblies are a dynamic biological response to cellular stress, limiting mRNA translation initiation to help the cell survive short-term stresses such as thermal, metabolic, and oxidative stress (3). Chronic stress granules form when the cell does not disperse the RNA-protein assemblies leading to cell death (4).

Objective:
To create models to study stress granule formation and disassembly in ALS in real time, in vivo.

Methods:
Zebrafish are the most suitable model organism for this study. Their high reproduction rate, a well characterised gene altering 'toolkit', and transparency in their embryonic and larval stages allow for a high number of samples to be genetically altered and screened over short time frames. Zebrafish share up to 70% of exons and major organs of interest with humans which should enable sound comparison at the cellular level of stress granule dynamics (5). In order to study stress granule formation, this project aims to link known stress granule associated proteins with fluorescent markers to visualise stress granule dynamics, in real time, in vivo, through confocal microscopy.

Results/Discussion:
We have injected stress granule reporters and visualised the formation of stress granules with some evidence to suggest they co-localised with ALS associated proteins. The goal for this research is to establish a model to understand stress granule dynamics and their role in ALS potentially aiding foundational steps toward better treatments or a cure.

References:
1. Bosco DA, Lemay N, Ko HK, Zhou H, Burke C, Kwiatkowski TJ, Jr, et al. Mutant FUS proteins that cause amyotrophic lateral sclerosis incorporate into stress granules. Human Molecular Genetics. 2010;19(21):4160-75.
2. Kedersha N, Ivanov P, Anderson P. Stress granules and cell signaling: more than just a passing phase? Trends in Biochemical Sciences. 2013;38(10):494-506.
3. Guzikowski AR, Chen YS, Zid BM. Stress-induced mRNP granules: Form and function of processing bodies and stress granules. WIREs RNA. 2019;10(3):e1524.
4. Wolozin B, Ivanov P. Stress granules and neurodegeneration. Nature Reviews Neuroscience. 2019;20(11):649-66.
5. Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, Muffato M, et al. The zebrafish reference genome sequence and its relationship to the human genome. Nature. 2013;496(7446):498-503.

IVV-35: Zebrafish Model of Mitochondrial Proteins CHCHD10 and CHCHD2

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Live Poster Session C, December 11, 2020, 12:05 PM - 12:50 PM

CHCHD10 is nuclear encoded mitochondrial protein whose cellular function remains to be fully understood. Several dominantly inherited missense mutations have been reported in its gene in patients with various neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). In the mitochondrial intermembrane space, CHCHD10 forms a complex of unknown function with CHCHD2, a protein that has been linked to rare cases of Parkinson's disease. In this study we present preliminary data that explores the biological function of the zebrafish ortholog of CHCHD10 and CHCHD2 as well as the pathological consequences of an analogous ALS-associated CHCHD10 missense mutation that was generated through gene editing using the CRISPR/Cas9 mutagenic system and homology directed repair. These models will permit studies examining ALS-associated Chchd10 variants in vivo, including the characterization of motor defects, neuromuscular junction (NMJ) denervation, as well as mitochondrial dysfunction which are believed to arise in patients with mutations in CHCHD10. Moreover, knock-out models will allow investigations that examine the consequences arising following the aberrant expression of the Chchd10-Chchd2 complex in vivo. Preliminary results suggest that loss of either Chchd10 or Chchd2 leads to reduced survival, weight, as well as muscle pathology in adult zebrafish. Compensation by Chchd10 has also been observed following Chchd2 loss, as well as reduced assembly of mitochondrial respiratory chains complex in Chchd10, and Chchd2 knock-out larvae was observed. Unravelling the cellular consequences resulting from the endogenous expression of a disease-associated Chchd10 variant as well as the aberrant Chchd10/2 complex expression will allow for a more thorough understanding of the biological mechanisms that culminate in neurodegeneration and may likely add to the complex and as of yet to be fully understood role that abnormal mitochondrial play in ALS as well as other neurodegenerative disease.