Session 1 Joint Opening Session

C1 ALS/MND: defining the disease

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Keywords: heterogeneity, diagnosis, disease subtypes

There is, perhaps, no greater challenge or opportunity currently in the field of motor neuron disease (MND) research than to ensure that the patient populations we study are affected with the same underlying condition. Understanding both the heterogeneity and the homogeneity within the patient populations we treat has become a critical prerequisite, especially in light of multiple failed treatment trials of the past.

Diagnostic certainty in patients with ALS/MND has historically been dependent upon well-established clinical criteria that have been continually refined. Despite well-established criteria, however, significant clinical variability still exists within the spectrum of ALS/MND. The extent of that variability has highlighted the need for alternative biochemical, imaging and genetic biomarkers to help ensure more homogeneous patient populations. No marker to date, however, has proven to define a similarly affected patient population. The clinical spectrum defined by a single gene mutation, a specific biochemical marker or a particular imaging index, remains very broad. This has resulted in uncertainty as to which disease associated marker or criteria is most valid to define a group of patients affected with the same condition, resulting from the same mechanism. We are currently confronting a paradoxical cross-roads with advancing technology yielding better clinical, biochemical, imaging and genetic tools while the disease states they define remain vast.

Heterogeneity is inherent in all of ALS; the disease presentation and its potential pathophysiology(s). Phenotypically, independent primary attributes of body region of onset, relative mix of upper motor neuron (UMN) and lower motor neuron (LMN) deficits, and rate of progression yield a multitude of combinations. Recent advances associating frontotemporal dysfunction in some patients with ALS/MND has resulted in both an opportunity to define a subgroup of patients as well as contribute to the overall disease variability. Genotypic pleiotropy and oligogenic effects are now well recognized and epigenetic influences provide further opportunities for disease heterogeneity.

In an effort to define patients who may respond to similar treatments resulting from similar pathophysiology, we are left with many clues but few conclusions. We have not yet developed a consensus on any criteria defining a disease subtype distinct in etiology from the others.

We no longer have the luxury of waiting for a leading treatment to shed light on a subpopulation of responding patients. The reality of the ALS/MND syndrome(s) as potentially separate disease entities cannot be ignored and might become our primary focus. The risk of maintaining our current momentum is greater than the alternative of exploring differences in the disease entity we strive to define.

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C2 The ‘lateral sclerosis’ half of ALS: corticospinal (‘upper’) motor neurons from Charcot to their molecular development, diversity, circuitry, and growth cones

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Keywords: upper motor neurons, corticospinal, development

Since Charcot’s description in 1869 and naming in 1874, ALS has been the coordinate degeneration of both corticospinal (‘upper’) motor neurons (CSMN; their axons in the lateral CST were observed as sclerotic, thus L[ateral] S[clerosis]) and spinal (‘lower’) motor neurons (SMN; ‘anterior horn cells’) that define ALS. Though not as absolutely purely motor system as long thought, before improved lifespan support enabled identification of cognitive/other involvement in ALS, these two neuron subtypes are still the defining and core, selectively vulnerable subtypes. Thus, it is critical to elucidate why these two quite distinct populations degenerate coordinately, in cortex and spinal cord, though developmentally born from distinct progenitor domains, with different neurotransmitter systems, synaptic types, and surrounding interneuron and astroglial types.

Why do variants in genes expressed in every neuron type, including hundreds-thousands in cortex, cause disease risk, with (relatively) selective vulnerability? What is common with subtypes involved in FTD that might clarify shared vulnerabilities with ALS? Why do involved neurons function so well in people who later develop ALS (eg Lou Gehrig), indicating lack of early dysgenesis? Might complexity of evolutionary advancement in primate-human corticospinal system partially explain fragility/selective vulnerability of component neurons? Length and/or metabolic demands alone cannot- sensory DRG neurons and many cortical projection neurons are similarly long, yet not similarly involved. Bulbar ALS affects shorter neurons rather than longer CSMN. Some SMN subtypes survive. Understanding these issues is necessary to grasp or model ALS, its causes, and routes to therapy.

I will address these questions primarily through a molecular developmental viewpoint. Given the immense diversity of CNS neuronal subtypes (particularly in cortex), and complexity and precision of functional circuits, degeneration of CSMN and SMN might be optimally understood via their development– potentially making them vulnerable later to ALS polygenics. I will begin with cortical projection neuron diversity, and combinatorially interacting developmental controls (in particular, for CSMN) that comprise a nested ‘molecular logic’ controlling key developmental processes– progenitor domains, partial fate restriction, subtype-specific differentiation, areal identity, implementation of circuit wiring by growth cones. I will discuss new approaches enabling identification of deep, subtype-specific growth cone protein and RNA regulatory networks underlying generation, and potentially underlying problems in maintenance, thus degeneration, of specific CSMN-SMN circuitry. I will discuss developmental links between ALS and FTD, and delineation of subsets of ALS/MND (eg bulbar, HSPs) based on their molecular development. Experimental questions, results, and speculations will be included together.

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C3 Synapse dysfunction of layer V pyramidal neurons precedes neurodegeneration in a mouse model of TDP-43 proteinopathies

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Keywords: TDP-43, dendritic spines, synapse

Background: TDP-43 is the major component of pathological neuronal inclusions in ALS, with mutations associated with disease onset and progression. Previous research has focused on the nuclear role for TDP-43 and toxicity of cytoplasmic aggregates; however, recent evidence indicates TDP-43 misprocessing, either as a result of mislocalisation or mutant gain/loss of function, may have an underappreciated pathological role at the synapse.

Objectives: To characterise synaptic pathology in the Thy1-YFP H × TDP-43A315T mouse model of ALS using regional immunohistochemistry, Western blots, electrophysiology and spine density analysis, and investigate alterations to neuroplasticity in disease using cranial window surgeries and 2-photon imaging.

Methods: Thy1-YFP H controls and Thy1-YFP H × TDP-43A315T mice were perfused with 4% paraformaldehyde over a time-course (P30, P60 and P90). Protein expression in the cortex was investigated using immunohistochemistry at P90 with pre- and post-synaptic
antibodies. Dendritic spines were imaged on a Zeiss LSM510 Meta confocal microscope and analysed using Neurolucida software. Cranial window surgeries were performed over the motor and somatosensory cortices of Thy1-YFPH and Thy1-YFPH x TDP-43<sup>A315T</sup> mice at P50 and P80, and imaged using a Scientifica 2-Photon microscope at P60 and P90, respectively, prior to analysis using Neurolucida software.

**Results:** Dendritic spine densities in the motor and somatosensory cortices of Thy1-YFPH mice increased from P30 (695 ± 49 p/mm P30 MC, 981 ± 56 p/mm P60 MC) to P60 (588 ± 50 p/mm P30 SSC, 889 ± 44 p/mm P60 SSC), peaked at P60 and were pruned by P90 (197 ± 9 p/mm P90 MC, 190 ± 3 p/mm P90 SSC). Comparatively, spine density was reduced in the motor cortex of Thy1-YFPH x TDP-43<sup>A315T</sup> mice at P60 prior to symptom onset (922 ± 24 p/mm control MC, 611 ± 18 p/mm TDP-43<sup>A315T</sup> MC), and in the motor and somatosensory cortices at P90 (188 ± 11 p/mm control MC, 113 ± 7 p/mm TDP-43<sup>A315T</sup> MC, 190 ± 8 p/mm control SSC, 147 ± 9 p/mm TDP-43<sup>A315T</sup> SSC), where cell loss was observed. Morphological spine analyses revealed a significant impairment in mature spine development within the Thy1-YFPH × TDP-43<sup>A315T</sup> motor cortex. Furthermore, dendritic spine alterations corresponded to lowered efficacy of synaptic transmission pre-symptomatically at P60.

**Discussion and conclusion:** The data suggest that morphological alterations to dendritic spine densities as mediated by TDP-43 mutations may be an early occurring disease event in ALS. Current work is now building upon these findings, looking to further probe disease-related changes to neuroplasticity in contrast to physiological conditions, by utilizing a cranial window imaging paradigm. Further research is needed to elucidate the mechanism behind synaptic alterations, to determine whether these early pathological events are an up-stream or down-stream event in the cascade that characterises this devastating disease.

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**C4 Mechanisms of FUS mediated ALS: insights from mouse genetics**

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**Keywords:** FUS, mouse models, fronto-temporal degeneration

**Background:** FUS mutations cause early onset, severe ALS. In ALS-FUS patients, cytoplasmic FUS aggregates are often associated with loss of nuclear FUS. Whether loss of nuclear FUS function, gain of a cytoplasmic function, or a combination of both lead to neurodegeneration remains elusive. Furthermore, while motor neuron-extrinsic mechanisms have been shown to participate in the pathogenesis of ALS-SOD (1), it remains unclear whether such mechanisms contribute to FUS-associated ALS.

**Objectives:** We sought to determine the relative contributions of gain and loss of function in ALS-FUS, and the contribution of motoneuronal expression of FUS expression to disease using newly generated conditional knock-in and knock-out mice.

**Methods:** We generated knock-in mice expressing mislocalized cytoplasmic FUS conditionally and complete FUS knock-out mice (1). We characterized the ALS and FTD-like phenotypes of homozygous and heterozygous knock-in and knock-out mice (2), and rescued the knock-in mutation in motor neurons through CRE mediated recombination. We performed RNAseq and RASLseq in spinal cord, brain and cerebral cortex.

**Results:** In homozygous state, both knock-in and knock-out FUS mice display perinatal lethality with respiratory insufficiency, and largely similar alterations in gene expression and mRNA splicing patterns, indicating that mislocalized FUS results in loss of its normal function. However, FUS knock-in mice, but not FUS knock-out mice, present reduced numbers of motor neurons at birth, which can be rescued by cell-specific CRE-mediated expression of wild-type FUS within motor neurons (1). Heterozygous FUS knock-in mice, but not mice heterozygous for a FUS null allele, develop similar pathology as ALS-FUS patients and a mild motor neuron phenotype. Most importantly, CRE-mediated rescue of the FUS mutation within motor neurons prevented degeneration of motor neurons, but only delayed appearance of motor symptoms. Further, we observed down-regulation of multiple myelin-related genes, and increased numbers of oligodendrocytes in the spinal cord supporting their contribution to behavioral deficits (2). Interestingly, heterozygous FUS knock-in mice also develop progressive atrophy of the frontal and temporal lobes associated with social disinhibition.

**Conclusion:** Loss of FUS is not sufficient to drive motor neuron degeneration. Cytoplasmic FUS mislocalization is necessary to trigger motor neuron loss. Mislocalized FUS triggers toxic events in both motor neurons and neighboring cells to elicit motor neuron disease and fronto-temporal related deficits.

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C5 Functional analysis of the ALS-associated miR-1825

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Keywords: mRNA, miRNA

Background: Increasing evidence suggests a dysregulation of miRNAs in ALS pathogenesis. Recently, we and others identified a significant down-regulation of miR-1234-3p and miR-1825 in the serum of sporadic ALS (SALS) patients (1,2). Additionally, down-regulation of miR-1825 was evident in the serum of familial ALS (FALS) cases (3). However, miR-1825 and miR-1234-3p are not well characterized and their mRNA targets have not been investigated so far.

Objectives: We aimed to determine if the reduced levels of miR-1825 and miR-1234-3p in serum of ALS patients reflect a systemic down-regulation of both miRNAs and to clarify the role of miR-1825 targets in ALS pathogenesis.

Methods: Using qRT-PCR we measured the relative levels of miR-1825 and miR-1234-3p in various post-mortem tissue (cortex, brainstem, spinal cord, liver, lymph node, skeletal muscle). Additionally, we identified the mRNA targets of miR-1825 by combining both proteomic (mass spectrometry) and transcriptomic (microarrays) profiling in HEK293 cells transfected with a miR-1825 mimic. Targets of miR-1825 relevant for ALS were validated using miRNA-pulldown and a luciferase approach.

Results: A significant down-regulation of miR-1825 was detected in post-mortem tissue of ALS patients while miR-1234-3p showed less prominent intracellular alterations. Several of the targets of miR-1825 were related to ALS and could be confirmed in vitro. Moreover, we will present a detailed evaluation of the most prominent miR-1825 target in an in vivo model as well as in post-mortem brain tissue of ALS patients.

Discussion and conclusion: Our results confirm previous studies showing a systemic down-regulation of miR-1825 in ALS (1,3). The main target of miR-1825 suggests a central role of microtubule in ALS pathogenesis.

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C6 Identification of target mRNA transported to axons by TDP-43

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Keywords: TDP-43, mRNA, axonal transport

Introduction: While abnormal deposition of TDP-43 is a hallmark in neurons of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with ubiquitin-positive inclusion bodies (FTLD-U), the pathogenic mechanism of the diseases involving TDP-43 is largely unknown. TDP-43 is an RNA-binding protein that supposedly regulates transcription, splicing, nucleocytoplasmic export and transport of mRNA. We hypothesized that the failure of mRNA transport to axons by TDP-43 is associated with neurodegeneration in ALS and FTLD-U, and aimed to clarify the function of TDP-43 by identifying the target mRNA for TDP-43-mediated axonal transport.

Methods: We detected mRNA decreased by shRNA-based TDP-43 down-regulation in axons of embryonic mouse cortical neurons using microarray analysis. We next analyzed the identified mRNA for binding and transport with TDP-43, localization, translation and function in axons. We also assessed the protective effects of overexpression of the identified mRNA in TDP-43-knocked-down cortical neurons, and in compound eyes of Drosophila overexpressing wild-type TDP-43.

Results: Down-regulation of TDP-43 expression decreased mRNA of ribosomal proteins in axons. TDP-43 binds and transports the mRNA through their untranslated region. These mRNA were translated locally, and overall ribosomal function was suppressed in axons by the decreased expression of TDP-43. Disrupted axonal extension in TDP-43-knocked-down cortical neurons was mitigated by overexpressed ribosomal proteins. Degeneration of the compound eyes in TDP-43 transgenic flies was also suppressed by overexpression of the proteins.

Discussion: In ALS and FTLD-U, neurodegeneration can arise from impaired protein synthesis in axons as a result of aggregation-mediated deficiency of transport of ribosomal protein mRNA by TDP-43. Enhancement of the target mRNA transport can be a novel strategy to treat ALS and FTLD-U.

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Session 2B Autonomy and Quality of Life

C7 Communication in serious illness: an evidence-based approach

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Keywords: clinician-patient communication, palliative care, medical decision-making

High-quality care for patients with ALS and other motor neuron diseases depends upon communication that is patient-centered, clear, and attends to the central role of emotion in the medical encounter. Good communication leads to a number of improved outcomes including greater adherence to therapy, higher patient satisfaction, decreased anxiety and depression, and care that is more consistent with patients’ goals. Unfortunately, in practice, communication frequently does not meet these standards and opportunities are lost to meet patients’ most basic needs. Also, a large body of evidence supports an approach to assess patients’ informational needs, attends to their emotion, and provides information in accordance with their preferences. Good communication can be deconstructed and conceptualized as a series of such discrete behaviours and skills. Specific techniques and cognitive roadmaps exist (and will be described) to help navigate these conversations, and to help patients align medical treatments to their values and goals.

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C8 Health status perspectives in amyotrophic lateral sclerosis

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Keywords: quality of life, ALS functional rating scale, health condition visual analogic scale

Introduction: Global perception of amyotrophic lateral sclerosis (ALS) patients’ own health status (HS) before the initial diagnosis has not been addressed before.

Methods: We recorded, at first visit, before diagnostic information: Visual analogical scale (VAS) of the EuroQol-5D; Revised ALS functional rating scale (ALSFRS-R), bulbar (ALSFRSb), upper limb (ALSFRSul), lower limb (ALSFRSl) and respiratory (RofALSFRS-R) subscores; Forced and slow vital capacity. Correlations were tested by Pearson’s correlation test. Variables were compared between groups defined by VAS median value. p<0.05 was considered significant.

Results: In 156 patients (91 spinal onset, 49 bulbar onset, 16 axial/respiratory onset; 95 males; mean onset age 63.9±13 years; mean disease duration 18.4±26.5 months). HS VAS was significantly lower in spinal-onset patients (p=0.047) and in spinal-onset females (p=0.027). Disease duration had no influence. HS VAS was moderately correlated with ALSFRS, ALSFRSul and ALSFRSl (0.4<r<0.5, p<0.01), weakly correlated with RofALSFRS-R in the whole population (r=0.171, p<0.05) and not correlated with ALSFRSb or the respiratory tests. ALSFRSb was similar between groups defined by HS VAS median value, but the other scores were significantly lower for lower HS values.

Conclusion: HS before diagnosis is mostly dependent on the perception of upper and lower limb function. A tool tailored to evaluate HS in bulbar-onset patients should be developed.

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C9 ALS patients with locked-in syndrome: quality of life, depression and medical decision making

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Keywords: QoL, locked-in, depression

Background: With appropriate therapeutic support, ALS patients may experience a locked-in syndrome (LIS). As interviews in these patients are strenuous and
time-consuming (mostly, the only means of communication is via eye-blink response to indicate ‘yes’/‘no’), extensive interviews on patients’ perspectives and wellbeing are rare.

**Objective:** To interview ALS LIS patients on their wellbeing and preferences to end life.

**Methods:** In total, \( n = 25 \) ALS patients in far advanced stages and with locked-in syndrome (14 male; age 58.0 ± 9.5 years; \( n = 3 \) NIV, \( n = 22 \) IV; \( n = 24 \) PEG) were interviewed on indicators of wellbeing global quality of life (QoL): ACSA (range −5 to +5), subjective QoL: SEIQoL (range 0–100%) and depression with ADF12 (range 12–48). Social support and burden of care were determined on a 4-point Likert scale. Furthermore, attitudes towards hastened death (SAHD) and preferences for hypothetical ideation to terminate therapeutic techniques (IV, NIV, PEG) in the future were assessed. Physical function was measured with ALSFRS-R (3.4 ± 4.7). Association of measures was tested with Spearman-Rho correlations (threshold of significance \( p < 0.05 \)).

**Results:** Subjective experience of social support (3.5 ± 0.7) and burden for the family (3.2 ± 0.8) were high. Global QoL was in a positive range (ACSA 0.23 ± 3.1) and subjective QoL was satisfactory (SEIQoL 70.4 ± 18.4). Overall mean depression rate was in a low range (ADF12 26.3 ± 5.7) and only \( n = 7 \) patients presented with clinically relevant symptoms of depression. Wish for hastened death was low (SAHD 4.4 ± 3.8) and only one patient presented with a clinically relevant score. Physical function was not associated with depressiveness \( (r = 0.7, \ p = 0.07) \) or subjective QoL \( (SEIQoL \ r = 0.2, \ p = 0.4) \). There was a trend towards severely impaired patients showing a higher global QoL (ACSA \( r = -0.4, \ p = 0.08 \)). The lower the physical function, the lower was the wish for hastened death \( (r = 0.5, \ p = 0.04) \). Preferences to hypothetically terminate NIV, IV or PEG in the future were low and only \( n = 7 \) for IV and PEG and \( n = 9 \) for NIV would hypothetically consider doing so.

**Discussion:** In LIS state, ALS patients’ QoL was in a satisfactory range and clinically relevant depressiveness was rare. Physical function was not associated with wellbeing. Preferences for termination of therapeutic devices in the future were reported in a subset of patients only. Wish for hastened death was low and in the range of patients in early stages of the disease as previously reported, indicating a constantly low level throughout the disease. These findings provide valuable information for our understanding of living with ALS up to locked-in syndrome with high wellbeing if social support is provided.

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**C10 A systematic review of decision making among patients and their family in ALS care**

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**Keywords:** decision making, family processes, supportive care

**Background:** ALS patients and their families face complex decisions about healthcare interventions from disease onset to bereavement. Integration of family into the decision-making process can impact on how patients make decisions about care. However, little is known about how ALS patients and their family interrelate in decision-making processes pertaining to care.

**Objectives:** To examine the ALS patient/family relationship in the decision-making process; to ascertain how ALS patients and their families can shape one another’s decisions pertaining to care.

**Methods:** A systematic review of peer-reviewed empirical research published in full and in English between January 2007 and January 2017, related to patient and family decision-making in ALS care, was undertaken. Databases searched included: Medline; CINAHL; AMED; PsycINFO; PsycARTICLES; and Social Sciences Full Text. Search words used included: amyotrophic lateral sclerosis; motor neurone disease; patient; family carers; caregivers; family carers; carers; family members; decision making; decision making process; preferences; perceptions; experiences; care; health care; services; and palliative care. A narrative synthesis of the evidence was conducted (1).

**Results:** 55 papers from 47 studies that captured decision making in care for ALS patients and their family members were extracted. Findings were classified into the following categories: sourcing information about ALS; life-prolonging and life-ending interventions; advance care planning; genetic testing and family reproduction; support seeking; and family reliance and obligation. Only one prospective population-based study was identified and over half of the studies extracted were qualitative in design. Few studies used patient/family decision-making scales in data collection. The family viewpoint was captured primarily from family members with direct care-giving duties. Patients’ cognitive status was not routinely assessed. Studies which did screen patients for cognitive impairment excluded patients who had clinically overt dementia or severe cognitive impairment. Findings showed that family directly or indirectly impacted on whether patients requested, accepted or declined interventions. ALS patients’ and family caregivers’ decisions about care were shaped by their desire to minimise distress for one another and by their mutual obligations towards each other. The concerns that ALS patients, their family caregivers, and family members at known risk of ALS had for the wider
family, was a key factor that influenced decision-making processes within the ALS family unit.

**Discussion and conclusions:** Attention to family member roles beyond the primary caregiver role is needed. Strategies that support the cognitively-impaired ALS patient in the decision-making process need development. Identification of the substantive domains in which ALS patients and their families support one another in the decision-making process would enable the formulation of patient/family decision-making tools in ALS care.

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C11 RNA-binding proteins and nucleocytoplasmic transport defects in amyotrophic lateral sclerosis

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Keywords: nucleocytoplasmic transport, stress granules, phase transitions

A common denominator of neurodegenerative diseases is the presence of protein inclusions with a protein composition that is typical for each type of these diseases. In amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) the predominant pathological species detected in these aggregates are RNA-binding proteins. These proteins are depleted from their normal nuclear localization, mislocalize to the cytoplasm and ultimately form macroscopic aggregates. These key pathological features suggest a stepwise mechanism.

The first question is how this pathological cascade is initiated. In most patients, and especially in sporadic cases, this is not yet clear. Recent data on C9orf72 ALS/FTLD disease models point at a crucial role for the nuclear transport system in this toxicity. We observed that the arginine-containing dipeptide repeat proteins (DPRs), which can be translated in a non-ATG mediated way from the hexanucleotide repeat expansions in C9orf72, can cause toxicity. This toxicity is modulated by interfering with the expression of importins, exportins, Ran-GTP cycle regulators, and nuclear pore components.

Once mislocalized into the cytoplasm, RNA-binding proteins can become part of stress granules, a process in which the arginine-rich domains of these proteins play a crucial role. We recently observed that the arginine-rich DPRs can also undergo liquid–liquid phase separations. Moreover, these DPRs can induce phase separation of a large set of proteins involved in RNA metabolism and they are also able to induce spontaneous stress granule assembly in cells. These stress granules are generally considered to be the stepping stone towards the pathological aggregates containing RNA-binding proteins.

Altogether, both arginine-rich DPRs and RNA-binding proteins seem to play an important role in the pathogenesis of C9orf72 ALS/FTLD. Moreover, disturbances in nucleocytoplasmic transport could initiate a cascade of events ultimately leading to the formation of cytoplasmic aggregates containing RNA-binding proteins.

C12 The nuclear pore complex is compromised in SALS and ALS/FTD

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Keywords: nuclear pore complex, nucleocytoplasmic transport, SALS

An expanded hexanucleotide repeat (HRE) [GGGGGC] in intron 1 of the C9orf72 gene is the most common genetic cause of familial and sporadic amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Until recently, very little was known about the underlying mechanisms by which this expanded repeat causes neurodegeneration until five independent laboratories published three papers including one from our own group simultaneously showing that dysfunction in nucleocytoplasmic transport (NCT) may be a fundamental pathway for C9orf72 ALS-FTD pathogenesis.

In order for any cell to function properly, it is imperative that RNA and protein are efficiently and selectively exchanged between the nucleus and the cytoplasm. This critical task is achieved by the ~2000 nuclear pore complexes (NPC) that span the entire nuclear envelope and serve as the main gateway of the nucleus. Each NPC consists of multiple copies of 30 different proteins called nucleoporins (NUPs) and mutations in various NUPs result in tissue-specific diseases. Interestingly, some of the longest-lived proteins in the mammalian brain are specific NUPs and may represent the ‘weakest link’ in the aging proteome. Finally, one-third of all NUPs contain multiple repeats of hydrophobic phenylalanine-glycine (FG) and these FG-NUPs are found along the entire transport route of the NPC. FG-NUPs create an entropic barrier to diffusion through the NPC, are highly dynamic, have short residence times, interact directly with transport receptors, control nucleocytoplasmic transport, and determine the pore permeability limit.
We now present data that the NPC is not only compromised in models of C9orf72 but also SALS, suggesting that NPC dysfunction may be a common insult and pathogenic mechanism in the majority of ALS. More specifically, we have surveyed the majority of NUPs in transgenic and BAC C9orf72 mice, iPSC neurons, and human post-mortal brain tissue using IF, IHC, super resolution imaging, Western blot, shRNA, and proteomic analysis. We have identified a unique set of NUPs and transport machinery with critical and disease relevant functions that are consistently affected across not only models of C9orf72 but also SALS, with the majority of these being components of nuclear import machinery that have FG-repeats. Knockdown of FG-NUPs disrupts the ran gradient responsible for fueling nucleocytoplasmic transport and also causes cytoplasmic mislocalization of pTDP43 and critical non-FG-NUPs. General inhibition of nuclear import causes cytoplasmic mislocalization of FG-NUPs and pTDP43 as well as stress granule formation. Finally, all these deficits can be rescued when treating with a potent small molecule inhibitor of nuclear export.

These data suggest that NPC dysfunction may be a common insult in the majority of ALS and that disruption of FG-containing NUPs/transport receptors involved in nuclear import may be the first ‘dominoes’ to fall in the disease cascade.

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C13 Safety and efficacy of SRSF1-dependent nuclear export inhibition of C9ORF72 repeat-transcripts: moving towards therapies

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Keywords: C9orf72, SRSF1, mRNA nuclear export

Background: Hexanucleotide repeat expansions in the first intron of the C9orf72 gene are the most common known cause of ALS and FTD (1,2). They exert neurotoxicity through complex mechanisms involving haploinsufficiency, RNA-repeat sequestration of proteins and production of dipeptide repeat proteins (DPRs) by repeat-associated non-ATG (RAN) translation. We recently showed that the nuclear export of sense and antisense repeat transcripts is driven by sequestration of the nuclear export adaptor SRSF1 (3). Inhibiting the nuclear export of repeat-transcripts and the subsequent RAN-translation of DPRs through partial depletion of SRSF1 prevents neurodegeneration in patient-derived neurons and C9orf72-ALS Drosophila (3). This intervention also rescues associated locomotor deficits highlighting a novel promising therapeutic strategy of neuroprotection.

Objectives: This study aims at (1) evaluating the safety of antagonizing SRSF1 using transcriptomics approaches in neuro-protected C9orf72-ALS patient-derived neurons and Drosophila and (2) test whether SRSF1 cell permeable inhibitory peptides can respectively be used to alleviate neurotoxicity and locomotor deficits in C9orf72-ALS patient-derived neurons and Drosophila.

Results and discussion: Our transcriptomics data show that antagonizing SRSF1 does not cause widespread dysregulation of gene expression or of the bulk mRNA nuclear export in agreement with the recent finding that other factors of the SRSF family of proteins can functionally compensate the RNA-processing and nuclear export adaptor functions of SRSF1 (4). Interestingly, cell death, synaptic transmission, immune and stress response pathways are up-regulated in C9orf72-ALS compared to control neuron or Drosophila head samples while the depletion of SRSF1 which confers neuroprotection reciprocally leads to manipulation of immune response, cell death and synaptic transmission. On the other hand, transcripts encoding proteins implicated in neuronal polarity, locomotion and immune response are differentially expressed in control versus neuro-protected C9orf72-ALS Drosophila in agreement with motor function rescue. Importantly, we also show that the partial depletion of SRSF1 specifically inhibits the nuclear export of pathological C9orf72 repeat transcripts while it has no effect on the splicing or nuclear export of the wild-type C9orf72 transcripts that encode the C9orf72 protein. Finally, we report that SRSF1 cell permeable peptides that target the nuclear export machinery leads to reduced DPR expression and rescues locomotor deficits C9orf72-ALS Drosophila thereby providing an additional promising therapeutic strategy of neuroprotection.

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C14 Phase separation of FUS is suppressed by the nuclear import receptor transportin and FUS arginine methylation

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Keywords: FUS arginine methylation, phase separation, transportin

Background: Cytosolic FUS aggregates are a pathological hallmark in a subset of patients suffering from amyotrophic lateral sclerosis (ALS) or frontotemporal dementia (FTD) (1). A key step that is disrupted in these patients is nuclear import of FUS mediated by the nuclear import receptor transportin (also known as Karyopherin-b2). Mutations in the nuclear localization signal (NLS) of FUS that weaken transportin binding cause familial ALS (2). Transportin aggregates (3) as well as defects in arginine methylation of FUS, which regulates the FUS-Transportin interaction, are observed in FTD-FUS patients (4).

Objectives: Recently, it has been proposed that FUS and related RNA-binding protein aggregates form via aberrant phase transitions, i.e conversion from a liquid-like to a solid-like, aggregated state (5). We sought to investigate how phase separation and aggregation of FUS is influenced by arginine methylation of FUS-RGG domains, or the interaction with transportin.

Methods: Recombinant full-length FUS or the C-terminal FUS-RGG-NLS domain was purified from bacteria and arginine methylated in vitro. Phase separation/aggregation was analyzed in various in vitro assays (e.g. droplets visualized by microscopy, turbidity measurements, FRAP measurements, sedimentation analysis) as well as a cellular assay utilizing semi-permeabilized HeLa cells.

Results: We show that both transportin and arginine methylation have a crucial function beyond nuclear import and suppress liquid-liquid phase separation and aggregation of FUS via the C-terminal RGG3-PY-NLS domain. We further demonstrate that transportin can suppress aggregation of FUS in the cytoplasm of semi-permeabilized cells. Our data show that ALS-associated FUS mutations render the protein less sensitive to the chaperone activity of transportin and that loss of arginine methylation, as seen in FTD-FUS patients, promotes phase separation and aggregation of FUS.

Discussion and conclusion: Our findings reveal two novel mechanisms of liquid phase homeostasis that are disrupted in FUS-associated ALS and FTD, supporting the view that phase separation and liquid-to-solid phase transition of FUS are at the heart of the pathogenic process.

Acknowledgements: This work was supported by the German Research Foundation (DFG) by the Emmy Noether grant DO 1804/1-1 and the Munich Cluster for Systems Neurology (SyNergy, EXC 1010) as well as the Investment Fund and Junior Research Fund of the LMU Munich.

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C15 Enhancing neurological care through telemedicine

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Keywords: telemedicine, healthcare, telehealth

In many ways, current care models for many chronic neurological conditions could not be designed worse. Individuals with ALS and other motor neuron diseases often have limited mobility and are primarily living in suburban and rural areas. Current care models are primarily located in urban centers, and require mobility to access care. By using technology, we can increase access to care for individuals with ALS and other chronic neurological conditions.

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C16 Will telehealth revolutionize clinical care for ALS patients?

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Keywords: telehealth, precision medicine, big data

Technology is changing the manner in which patients manage their health and interface with the medical community, a revolution as dynamic as the integration of cell phones and social media into society. The miniaturization and reduction in costs of technologies to monitor your own health is integrated with user friendly data management and data analysis solutions. This revolution is facilitating the ability of patients to monitor and interpret changes in their health, and act on these in conjunction with their physicians in complex scenarios. These advances, often termed Precision Medicine, are becoming mainstream trends in society.

The implementation of Precision Medicine into a patient’s health management experience depends on the successful integration of two distinct processes: data aggregation and data interpretation.

Data aggregation consists of family and medical history; genetic data; biomarker data at the DNA, RNA and protein levels; lifestyle information; medications; supplements; and longitudinal data from wearable technologies and biosensors.

Data interpretation is a dynamic process by which patients, physicians, and data analysts (geneticists and computational biologists) model changes in a patient's health based on the aggregation of these data.

The value proposition of Precision Medicine for the patient, clinical care team and health care system is optimized patient care, the merits of which are: (1) successful disease prevention; (2) accelerated disease intervention; (3) effective disease management.

Telehealth empowers a patient to be actively involved in assessing and managing their health care decisions. The ability of patients and physicians to review and assess health status remotely and in real time decreases the frequency of clinical visits, reduces clinical costs and reserves the allocation of clinical resources for patients who need them most.

In ALS there are many Precision Medicine and telehealth programs established across the globe. The ALS Therapy Development Institute's Precision Medicine Program seeks to gain new insight into the mechanisms of ALS disease onset and progression. This is being accomplished by obtaining a comprehensive background on each participant's lifestyle and medical history.

These data are integrated with each participant's genetic data obtained by genome sequencing. The progression of disease is assessed by collecting data on wellness using the ALS Functional Rating Scale, quantitative tracking of movement using accelerometers, and changes in speech using voice recordings. New drug development tools will be created from patient-derived cells to support identification and development of novel, focused drug discovery screens.

A critical component of the program embracing the future of telehealth is providing all participants access to their data via an online portal. The program has enrolled 350 participants with IRB approval to enrol 750 participants. We will report preliminary data on longitudinal voice recordings and accelerometer data along with self reported ALS FRS from the current cohort.

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C17 BrainGate: toward restoring communication and mobility

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Keywords: brain-computer interface, neurotechnology, motor cortex

Intracortically-based brain-computer Interfaces (iBCIs) are poised to revolutionize our ability to restore lost neurologic functions (1–4). By recording high resolution neural activity from the brain, the ‘intention’ to move one’s hand can be detected and decoded in real time, potentially providing people with ALS with restored or maintained ability to control external devices. A multi-site pilot clinical trial of the investigational BrainGate system is assessing the feasibility of people with tetraplegia from ALS/MND controlling a computer cursor and other devices simply by imagining the movement of their own arm or hand. In this presentation, we will review progress in iBCIs clinical trials, as well as challenges and opportunities for restorative neurotechnologies in research and clinical practice.

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Session 4A RNA and Stress Response

C18 Neuroprotective effects of angiogenin-induced tRNA cleavage

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Keywords: angiogenin, tRNA, stress

Angiogenin (ANG) is a stress-activated ribonuclease that promotes the survival of motor neurons. Ribonuclease inactivating point mutations are found in a subset of patients with amyotrophic lateral sclerosis. We recently showed that ANG cleaves tRNA within anticodon loops to produce 5’- and 3’-fragments known as tiRNAs.选了5’-tiRNAAs1 (eg tiRNAAla and tiRNAAsys) co-operate with the translational repressor YB-1 to displace eIF4F from m7G-capped mRNA, inhibit translation initiation and induce the assembly of stress granules (SGs). Here we show that tiRNAAla binds the cold shock domain of YB-1 to activate these translational reprogramming events. We discovered that 5’-tiDNAAla (the DNA equivalent of 5’-tiRNAAla) is a stable tiRNA analogue that displaces eIF4F from capped mRNA, inhibits translation initiation, and induces the assembly of stress granules. 5’-tiDNAAla assembles a G-quadruplex structure that allows it to spontaneously enter primary motor neurons and trigger a neuroprotective response. Our results introduce 5’-tiDNAAla as a lead compound for the development of a new class of neuroprotective drugs.

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C19 Mild chronic stresses sensitise neurons to the acute strong stress by reducing their capacity to maintain stress granule assembly

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Keywords: stress granules, FUS, phospho-eIF2alpha

Background: Various acute stresses may play a role of the ‘second hit’ triggering progression of a dormant neurodegenerative process in neurons already affected by a ‘first hit’, a malfunction of disease-associated proteins. Timely assembly and disassembly of stress granules (SGs) is an important mechanism of cell survival in conditions of acute stress, and aberrant SG response is believed to contribute to the development of neurodegenerative changes in a wide range of conditions, particularly in amyotrophic lateral sclerosis (ALS). Compromised phosphorylation of eIF2alpha, a key regulator of SG assembly and disassembly is often observed in neurons of patients with neurodegenerative diseases as well as in various models of neurodegeneration. It is also known that pathological protein aggregation can induce some aspects of stress response in affected neurons without triggering of all-out stress response characterised by translational arrest and SG formation.

Objectives: The principal aim of our study was to investigate if a mild chronic stress that can be caused by malfunction of certain ALS-associated proteins affected cell response to acute stress.

Methods and results: In a model of FUS overexpression we have shown that accumulation in the cytoplasm of pathogenic forms of ALS-associated RNA-binding protein FUS leads to the formation of unconventional RNA granules, which negatively affects the assembly and/or maintenance of SGs and Processing Bodies (PBs). Similar granules were observed in human cells with CRISPR/Cas9-edited endogenous FUS gene. Using several model systems, including ES cell-derived human neurons and live mice, we have demonstrated that elevated p-eIF2alpha level in cells experiencing transient or persistent mild stress impairs the maintenance of SG assembly following acute severe stress, creating a situation of SG partial loss-of-function. Up-regulation of PP1 phosphatase regulatory subunits has been identified as a mechanism behind it. Results of histopathological analysis of ALS patients spinal cord sections were consistent with the results obtained in the model systems.

Discussion and conclusions: We hypothesise that changes of neuronal physiology instigated by genetic or environmental factors can be tolerated for a long time but still cause persistent mild stress in affected neurons, preconditioning them to a defective and therefore deleterious response to any kind of acute, SG-inducing stress.

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C20 Dynamics and nature of inclusions of TDP-43 and its isoforms

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Keywords: TDP-isoforms, stress granules, FRAP

Background: Typical hallmarks of ALS are pathological intracellular protein aggregates containing the TDP-43 protein. It has been shown that TDP-43 also associates with stress granules (SGs) and processing bodies (PBs), cytoplasmic RNA and protein containing inclusions of stalled translation, formed in response to cellular stress. Several studies have described lower molecular weight species of TDP-43 that correspond to N- or C-terminally truncated isoforms of the full length protein. It has been proposed that these truncated isoforms possess increased cytotoxic features and may contribute to cell death in ALS.

Objectives: Here, we investigated the role of TDP-43 and its N-terminally truncated isoforms TDP-25 and TDP-35 as well as C-terminally truncated isoforms TDP-272 and TDP-298 regarding formation and dynamics of SGs and PBs. Furthermore, we analyzed the link between these highly dynamic RNA granules, irreversible protein aggregation and cytotoxic properties of TDP-isoforms.

Methods: In immunofluorescence assays we labeled cells transiently transfected with EGFP-conjugated TDP-isoforms with the SG marker Tia-1 and PB marker DCP1A. Live cell imaging of cells transiently co-transfected with EGFP-conjugated TDP-isoforms and RFP-Tia-1 was used to study the formation and dynamics of inclusions containing different TDP-isoforms. For this purpose, we conducted FRAP (Fluorescence Recovery after Photobleaching) experiments to analyze the kinetics of inclusions formed by TDP-isoforms. Expression of TDP-isoforms in living cells also provided us with information about their cytotoxicity in relation to inclusion dynamics.

Results: We observed that TDP-35 and in some cases full length TDP-43 colocalized with Tia-1 while TDP-25, TDP-272 and TDP-298 could not be associated with SGs. Furthermore, only in cells transiently transfected with TDP-35 some of the formed inclusions were additionally labeled by PB marker DCP1A indicating that TDP-35 but not other TDP-isoforms may be involved in RNA degradation processes. Formation of TDP-35 inclusions further led to rapid cell death of transfected cells. Comparable to live cell imaging and FRAP studies of Tia-1, we observed EGFP-conjugated TDP-35 to be highly dynamic in forming and re-forming SG in transfected cells. FRAP studies of TDP-272 and TDP-298 showed no recovery of fluorescence after photobleaching, demonstrating that inclusions formed by C-terminally truncated TDP-isoforms are less dynamic than TDP-35 in SGs.

Discussions and conclusion: In this study we thoroughly examined several isoforms of TDP-43 and their role in RNA granule formation and dynamics and their effect on cell survival. We found that these isoforms are associated with different types of inclusions displaying distinct properties regarding size, formation kinetics and cytotoxic effects. Unravelling the link between these inclusions and cytotoxicity of TDP-isoforms could provide useful insights in TDP-related neuronal cell death in ALS.

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C21 Role of RNA G-quadruplex structures in the molecular pathology of C9orf72-amyotrophic lateral sclerosis

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Keywords: C9orf72, RNA, helicases

Background: The most common cause of familial or sporadic amyotrophic lateral sclerosis is a large G4C2 repeat expansion in the first intron of the C9orf72 gene. G4C2 repeat-containing RNA accumulates in nuclear foci or is exported to the cytoplasm for repeat-associated non-AUG (RAN) translation into potentially toxic dipeptide repeat proteins. Polyadenylated C9orf72 RNA species retaining the repeat-containing intron and in which downstream exons are spliced correctly are potential substrates for RAN translation. The G4C2 sequence forms highly stable RNA G-quadruplex structures which may initiate the formation of RNA foci and processing of the transcript from the expanded allele.

Objectives: The aim of this work is to determine the role of RNA G-quadruplex structures on the pathomechanism of C9orf72 ALS. The specific objectives are to investigate the consequence of interfering with RNA G-quadruplex structures on the molecular signatures of C9orf72 ALS, including RNA foci and processing of C9orf72 transcripts.

Methods: Lymphoblastoid cells and cortical neurons derived from induced pluripotent stem cells (iPSCs) from heterozygous C9orf72 G4C2 expansion carriers and control individuals were treated with the cationic porphyrin, TMPyP4. Nuclear foci were detected by fluorescence in situ hybridisation (FISH) using a 5’-TYE locked nucleic acid (LNA) probe. RNA was extracted from control and treated cells and analysed for intron retention by end-point RT-PCR. For co-localisation of RNA foci with helicases, HEK293T cells were transfected with (G4C2)72 and analysed by combined FISH-immunofluorescence.

Results: Lymphoblastoid cells and neurons differentiated from iPSCs (iPSNs) from expansion carriers display nuclear foci formed by G4C2 repeat RNA. Cells treated...
with TMPyP4 (50–100 μM) for 48 h display a decrease in the number of RNA foci of up to 36%. Analysis of poly(A)^+ RNA from TMPyP4-treated lymphoblasts showed that the treatment promoted splicing of the repeat-containing intron. DHX9 and DHX36 are two helicases able to bind to and resolve RNA G-quadruplex structures (1). Western blotting analysis of iPSSNs demonstrated a reduction of DHX9 and DHX36 levels in cells derived from expansion carriers compared to controls. In addition, DHX9 is recruited into intranuclear foci formed by (G_4C_2)_72 RNA in HEK293T cells.

**Discussion and conclusions:** These results suggest that the G-quadruplex structures formed by expanded G_4C_2 RNA are involved in the processing of the repeat-containing intron and in the formation of RNA foci. As sequestration of RNA binding proteins by RNA foci is likely to be a main driver of toxicity, interfering with G-quadruplex structures offers novel ways of dissecting the pathomechanism of C9orf72 ALS and represents an innovative and promising therapeutic strategy.

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C22 Masitinib as an add-on therapy to riluzole is safe and effective in the treatment of ALS

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Keywords: masitinib, kinase inhibition, phase III clinical trial

Background: Masitinib, an oral tyrosine kinase inhibitor, appears unique among other ALS-developmental drugs, exerting neuroprotection by simultaneously targeting microglia, macrophage and mast cell activity, both in CNS and PNS.

Objective: Evaluate masitinib plus riluzole (100 mg/kg) in amyotrophic lateral sclerosis (ALS).

Methods: ALS patients received riluzole plus masitinib 3.0 mg/kg/d (M3.0), 4.5 mg/kg/d (M4.5), or placebo (1:1:1) up to 48 weeks. Two groups were defined according to pre-randomization ALSFRS-R progression: ‘Normal Progressor’ (NP) of less than 1.1 points/month, and ‘Faster Progressor’ of at least 1.1 points/month; thereby, reflecting the inherent heterogeneity of phenotype and disease progression within the overall population. Primary endpoint was absolute change in ALSFRS-R from baseline to week 48 (ΔALSFRS-R) in NP patients at M4.5. Missing data were handled via LOCF with sensitivity analyses based on imputation methodology incorporating all patients (ITT population). Secondary endpoints included progression free survival (PFS), progression being defined as ALSFRS-R deterioration of greater than nine points from baseline or death, ALSAQ40, and FVC. Safety analysis included patients administered at least one dose of study drug.

Results: 394 patients (‘Normal + Faster Progressor’ cohort); were randomized to the M4.5 (130), M3.0 (131) and placebo (133) arms. The NP cohort comprised 330 patients: 106, 110 and 114, respectively. For the primary endpoint analysis (M4.5 in NP), masitinib showed a significant benefit in ΔALSFRS-R over placebo with a least-square means difference (LSM) of 3.4 (9.2 vs. 12.6); 95% CI 0.6–6.1; p = 0.0158. In terms of ALSFRS-R slope, masitinib showed a clinically meaningful retardation of 27% (0.77 vs. 1.21 points/month). All sensitivity analyses were positive, with p less than 0.02 according to imputation methodology. Masitinib also demonstrated benefit over placebo in the secondary variables, significantly improving median PFS by 25% (20 vs. 16 months, p = 0.0159); ALSAQ40 by 28.5% (ΔLSM of 19.4 vs. 27.2, p = 0.0078); and FVC by 22% (ΔLSM of 26.0 vs. 33.4, p = 0.0332). For M3.0 in NP, masitinib showed benefit over placebo for ΔALSFRS-R = 2.73 (-8.6 vs. -11.3, a 24% improvement, p = 0.0661); ALSAQ40 (ΔLSM of 15.6 vs. 23.7, a 34% improvement, p = 0.0057); and FVC (ΔLSM of -23.1 vs. -27.9, a 17% improvement, p = 0.1662). No significant difference was seen between treatment arms for analysis according to the ‘Normal + Faster Progressor’ cohorts.

Post-hoc analysis selecting patients with less than 24-month duration of illness and score for each ALSFRS-R item of at least 2, showed enhanced treatment-effect in terms of ΔALSFRS-R, with a ΔLSM of 4.5, corresponding to a 42% improvement, p = 0.0176. This indicates greater benefit is possible with early treatment. Common (greater than 10%) adverse events (AEs) with masitinib in the NP cohort were rash, nausea, diarrhea, and weight loss. Frequency of AEs, serious AEs, and severe AEs (placebo versus M4.5 and M3) was, respectively: 79% vs. 90% and 84%; 20% vs. 28% and 18%; 18% vs. 24% and 17%.

Discussion and conclusion: Masitinib 4.5 mg/kg/d demonstrated a significant therapeutic benefit with acceptable safety in ALS patients with a baseline ALSFRS-R progression rate of less than 1.1 points/month.

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C23 VITALITY-ALS: results of a phase III trial of tirasemtiv, a fast skeletal muscle troponin activator, as a potential treatment for patients with ALS

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Keywords: fast skeletal muscle activator, clinical trial
Background: ALS primarily causes progressive muscle weakness, culminating in worsening respiratory failure and death. Tirasemtiv is a fast skeletal muscle troponin activator (FSTA) that has shown the potential to preserve slow vital capacity and strength over three months in a large phase Ib trial (BENEFIT-ALS), although the primary endpoint (change from baseline in ALSFRS-R) was not met in this study. Limitations of BENEFIT-ALS included the short study duration, the lack of defined dose exploration, and a rapid dose escalation resulting in the possibility of suboptimal tolerability. However, the clear signal on muscle strength and slow vital capacity (SVC) supported a pivotal study. VITALITY-ALS was designed to confirm the results of BENEFIT-ALS on SVC and strength over a longer duration, to extend these findings to other clinical measures of potential benefit, to learn more about dose response characteristics, and to maximize tolerability by implementing a slower dose escalation.

Objectives: To assess the efficacy of tirasemtiv versus placebo on respiratory function and other clinical aspects of function in patients with ALS.

Methods: VITALITY-ALS is a multinational, double-blind, randomized, placebo-controlled, parallel group study with tirasemtiv treatment up to 54 weeks in patients with ALS. Following two weeks (compared to one week in BENEFIT-ALS) of open-label tirasemtiv (125 mg BID), participants who tolerated tirasemtiv were randomized 3:2:2:2 to placebo or one of three target total daily dose levels of tirasemtiv (250, 375 or 500 mg). Eligible participants with a diagnosis ≤24 months and minimum SVC ≥70% predicted were enrolled from centers in North America and Europe. Randomized participants escalated their dose every two weeks to the target dose level or to their maximum tolerated dose (compared to weekly in BENEFIT-ALS). A single down-titration was allowed as necessary. The primary outcome measure is change in SVC from baseline to 24 weeks. Key secondary outcomes include quantitative muscle strength, the respiratory domain of the ALSFRS-R, time to a drop in SVC by ≥20%, time to assisted ventilation, respiratory failure or death, and time to a fall in SVC to ≤50% during all 48 weeks of double-blind, placebo-controlled treatment. ALSFRS-R and survival are also measured. A randomized withdrawal phase lasting 4 weeks followed the completion of 48 weeks of treatment, to evaluate both the possibility of sustained benefit or rebound decline.

Results: Data collection will be complete in October 2017. Primary results will be presented here.

Discussion and conclusions: VITALITY-ALS is a phase III trial designed to determine the efficacy and tolerability of tirasemtiv in ALS, with the primary outcome measure being change in SVC at 24 weeks, and key secondary endpoints evaluating multiple aspects of patient function measured at 48 weeks.

Acknowledgements: Cytokinetics Inc. funded the study.

C24 Efficacy, safety and tolerability study of 1 mg rasagiline in ALS – a prospective, randomized, parallel-group, double-blind trial

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Keywords: rasagiline, clinical trial

Rasagiline is a selective MAO-B inhibitor with symptomatic efficacy in Parkinson’s disease. Preclinical studies have demonstrated that rasagiline has neuroprotective effects in both in vivo and in vitro models, suggested to be related to its propargyl moiety rather than MAO inhibition.

The purpose of the trial was to assess the efficacy of 1 mg/d rasagiline as add-on to riluzole. The primary endpoint was survival, secondary endpoints were ALSFRS-R, SVC and quality of life measures.

252 patients were randomized, 75 patients died during the 18 months study period. The Intention to Treat (ITT) analysis showed no evidence for a survival benefit during the observation period of 30 months. No significant difference was seen for ALSFRS-R and the SVC. The safety profile of rasagiline was unremarkable during the entire observation period. Subgroup analyses and analyses after genetic profiling of the study population are underway.

In conclusion, this randomized trial of 1 mg rasagiline showed that this drug has no disease-modifying effect in ALS.

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C25 Ibudilast: bi-modal therapy with riluzole in early and advanced ALS patients

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Keywords: macrophage migration inhibitory factor inhibitors, toll-like receptor 4 antagonist, glial cell modulators

Background: Ibudilast, a phosphodiesterase type 4 Inhibitor, is effective in 2 ALS gene-based Drosophila models, has a safety profile permitting assessment of effectiveness for targeting etiological pathways at different (early–distal-axonopathy; late–microglial-activation) ALS stages.

Objective: Report on the effects of a double-blind (DB) study versus placebo, during phase Ib/IIa epoch on clinical endpoint responsiveness (ALSFRS-R scores, manual muscle strength measurements in limb and orofacial muscles, slow vital capacity, maximal inspiratory pressure, maximal voluntary ventilation, bulbar and limb timed functional tests, quality of life measures and survival) to these measures at the end of the 6-month Open Label Extension (OLE), Washout (WO) and Post-Washout (PWO) epochs in the Early (not requiring non-invasive ventilation (NIV)) Cohort (EC) and Advanced (requiring NIV) Cohort (ANC) amyotrophic lateral sclerosis (ALS) patients.

Design and methods: Randomized (2:1) delayed-start, bi-modal ibudilast + riluzole/placebo + riluzole-controlled/six-month DB phase Ib/IIa clinical trial epoch followed by a six-month OLE, two-week WO, 7–31-month PWO epochs to evaluate the feasibility/tolerability/safety and clinical endpoint responsiveness of ibudilast in 51 randomized EC (51) and ANC (19) subjects.

Results: EC-DB-43/51 completed-0-6mon; 8/51-stops (0-deaths on study drug; 4-study drug intolerance (intol); 4-unable to travel (utt)); EC-OLE-35/43 completed-6-12mon; 8/43-stops (1-death off study drug; 4-intol 4-utt) EC-WO:32/35 completed per protocol-12mon +2weeks; 3/35-stops (0-deaths off study drug; 3-utt); EC-PWO-29/51 subjects alive at OLE completion by last subject entered-15-37mon; (22/51-deaths off study drug post DB-OLE). Per-protocol (PP) completers (PPwoNIV14 mean = 34.8 mon (95% CI = 32.7 to 36.9); PPwNIV18 mean = 9.4 mon (95% CI = 26.4 to 32.3)) had better survival (p = 0.0004, Kaplan-Meier) than non-per-protocol (nPP) completers (nPPwNIV6 mean = 24.0 mon (95% CI = 16.0 to 32.0); nPPNIV13 mean = 20.5 mon (95% CI = 16.6 to 24.3]). Onset to diagnosis (PPmean32 = 10.7 mon (95% CI = 7.8 to 13.5); nPPmean19 = 10.6 mon (95% CI = 8.0 to 13.1)) and Diagnosis to Baseline (PPmean32 = 10.9 mon (95% CI = 6.2 to 15.6); nPPmean19 = 10.9 mon (95% CI = 7.1 to 14.5)) intervals are identical in both groups. Examination of 32/51 PP EC OLE-WO subjects two weeks subsequent to six months OLE treatment with ibudilast indicated significant loss of manual muscle testing strength in sentinel ALS muscles with no change in ALSFRS-R or SVC in this period. Data lock and statistical analysis are proceeding.

Conclusions: Ibudilast administration is feasible, tolerable and safe over 12 months in EC and ANC ALS subjects. PP completion was associated with lower mortality compared with nPP completion. Preliminary observations on potential survival effects in ALS subjects who tolerated ibudilast for the entire DB and OLE epochs need further evaluation and analysis to determine (1) how these observations might relate to randomization to active ibudilast/placebo during the DB epoch; (2) the relationship to delayed start of ibudilast; (3) the continuation of ibudilast during the OLE epoch; and (4) the employment of NIV and other treatments during the course of this clinical trial. Longitudinal PWO epoch assessment of information on survival and other clinimetrics may be employed to provide preliminary evidence concerning the potential benefit of short-term exposures to ALS treatments on the disease course. Novel analytics need to be developed to assess the potential effects of different treatments for ALS patients in the context of interrupted compared with continuous treatment protocols.

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C26 Towards more efficient clinical trial designs in ALS: lessons from the Edaravone Development Program

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Keywords: edaravone, study design, clinical endpoints

Background: Clinical trials in ALS have to date been challenging to design for a variety of reasons including heterogeneity of disease symptoms and rate of progression, and the limited application of clinical endpoints for measuring quality of survival. Moreover, clinical endpoints may not be responsive to treatment effects in all patients. In the MCI186-19 study (1), progression of disability as assessed by the ALS Functional Rating Scale-Revised (ALSFRS-R) was slowed in a study population selected for clarity of ALS diagnosis, preserved independent function, and documented advancing disease.

Objective: Using post-hoc assessments of CI186-19, we examined decline in ALSFRS-R scores and change in mean domain scores of ALS Functional Assessment Questionnaire (ALSAQ-40) and how favorable effects of edaravone treatment during 24 weeks of double-blind treatment could be detected.

Methods: The study population in (1) had been selected based on post-hoc findings from an earlier study MCI186-16, which had broader enrolment criteria. In the current post-hoc analyses of MCI186-19, a decrease in the ALSFRS-R score of ≥6 from baseline was defined as an event and the absence of that decrease was defined as a censored value for Kaplan Meier curves. A similar analysis was performed for an event defined as a decrease of ≥12 points. To analyze change in ALSAQ40 domains, ANOVA was performed in the full analysis population (placebo n = 64, edaravone n = 68) and last observation carried forward was applied to randomized patients.

Results: Over 24 weeks, time to ≥6-point ALSFRS-R decline analysis showed statistically significant differences between edaravone and placebo groups. Of the 68 placebo-treated patients, 33 declined by ≥6 points compared with the 69 edaravone-treated patients of which 23 declined by ≥6 points. Between-group comparisons were statistically significant (p = 0.0040 log-rank, p = 0.0126 general Wilcoxon test) Survival analysis for time to ≥12 point decline was also statistically different by both comparison methods. For each of the 5 domains of the ALSAQ-40, edaravone was descriptively favored over placebo at 24 weeks. These were physical mobility (–2.54 ± 1.39), activities of daily living/independence (–2.77 ± 1.37), communication (–0.73 ± 1.07) eating and drinking (–1.23 ± 0.46), and emotional functioning (–1.17 ± 1.44).

Conclusion: Both time-to-event analysis for ALSFRS-R decline and the individual domains of the ALSAQ-40 appear to be responsive to treatment effects of edaravone during 24 weeks of double-blind treatment. A study design with a well-defined patient population to reduce heterogeneity, and appropriate endpoints including functional decline and quality of life may be important in order to efficiently demonstrate a drug effect in 24 weeks.

Reference

1. The Writing Group on behalf of the Edaravone (MCI-186) ALS 19 Study Group. Lancet Neurol. 2017;16:505–12.

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C27 Antisense oligonucleotide based therapies for motor neuron diseases

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Keywords: antisense oligonucleotides, spinal muscular atrophy, RNA

Antisense oligonucleotides (ASOs) are synthetic, chemically modified nucleic acid analogs designed to bind to RNA by Watson-Crick base pairing and upon binding modulate the function of the targeted RNA. There are a variety of mechanisms by which ASOs can modulate RNA function dependent on the chemical design of the ASO, the type of RNA and where on the RNA the ASO is designed to bind. These include promoting the degradation of the target RNA and modifying intermediate metabolism such as splicing or polyadenylation. Both protein coding, as well as non-coding RNAs, can be targets of ASO based drugs, significantly broadening therapeutic targets for drug discovery compared to small molecules and protein based therapeutics. The recent approval of nusinersen (Spinraza™) as a treatment for spinal muscular atrophy (SMA) validates the utility of antisense drugs for the treatment of motor neuron diseases. This talk will highlight the key findings from the nusinersen clinical data in SMA, especially those findings that are relevant for the development of antisense technology for other motor neuron diseases. In addition, the progress in developing antisense drugs for other motor neuron diseases will be discussed.

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C28 Improving drug access to the CNS

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Keywords: antibodies, drugs, BBB

Delivery of many types of drugs to the CNS such as small organic molecules, antibodies, growth factors, enzymes and antisense oligonucleotides remains a huge problem because of the impermeable nature of the blood-brain barrier (BBB). Failure in expensive clinical trials of neurodegenerative diseases such as ALS is often ascribed to poor pharmacokinetics (PK) and CNS exposure meaning that the drug cannot interact with the relevant target and elicit a pharmacological and ultimately a clinical response. Consequently, patients are denied potentially life-changing medications. For small organic molecules, it is sometimes possible to alter the chemical structure to enhance CNS exposure without significantly altering activity against the drug target, but it is a risky strategy. For biological therapeutics, it is clear that brain exposure is normally 0.1% of plasma exposure due to the BBB and it is generally impossible to deliver therapeutic doses. This likely explains a slew of high profile clinical trial failures, which has turned the industry against CNS drug discovery. However, there are new approaches that could significantly alter the landscape. Receptor-mediated transporters in the luminal membrane of brain capillary endothelium offer an approach for the delivery of therapeutics to the brain. One of the best-characterized transporters is the transferrin receptor, which has been shown in a number of studies to offer the possibility of delivering drugs to the brain. The process has been termed the ‘Trojan Horse’ approach as the therapeutic drug is delivered to the brain via covalent coupling to a transferrin receptor antibody, which carries it across the BBB.

At Ossianix, we have used a combination of in vitro and in vivo phage display technology to isolate a panel of cross-species binders to the transferrin receptor 1 using synthetic single domain VNAR antibody libraries. At therapeutic doses (2 mg/kg) delivered by tail vein injection, high levels (>5% brain/plasma ratio) of a number of bispecific antibodies were found in the brain after 18 hours. This approach now offers the possibility of delivering therapeutic antibodies to the CNS and can also be applied to other drug classes.

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C29 Harnessing machine learning and artificial intelligence to identify novel ALS therapeutics

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Keywords: artificial intelligence, therapeutic, iNPCs

Rationale and hypothesis: Machine learning and artificial intelligence are transforming the process of scientific discovery by allowing scientists to see deeply into vast
research data sets and augment scientific intuition. The technology enables previously unimaginable scientific advances and has great promise in accelerating drug development where powerful AI tools are used to understand disease pathways, generate new hypotheses and identifying novel therapeutic targets and molecules. BenevolentAI used this technology to identify several compounds, including compound A, which may have therapeutic potential in ALS. We had previously developed a co-culture system where astrocytes from ALS patients induce motor neuron cell death, thus providing a pathophysiologically relevant readout for drug screening. We tested compound A for efficacy in these patient-derived co-culture models and in vivo using a mouse model of ALS.

Objectives: To validate the AI approach to compound identification using a high-throughput screening system using in vitro co-cultures of ALS patient-derived astrocytes and motor neurons, and in vivo mouse models of ALS.

Methodology: We used ALS patient-derived skin fibroblasts, and converted them rapidly and directly to induced neuronal progenitor cells (iNPCs), which were then differentiated into iAstrocytes or iMotorNeurons. The iAstrocytes were cultured in 384-well plates, and small molecules were delivered rapidly using an Echo550 liquid handler. Hb9-RFP-lentivirus-transduced human iMotorNeurons were then seeded in co-culture with the pre-treated human iAstrocytes. iMotorNeurons were then imaged every 24 hours using an INCELL analyser 2000, and the number of viable cells was counted using the Columbus™ analysis software. For the in vivo efficacy study, we dosed female G93A-SOD1 C57BL/6 mice orally once daily with compound A (10 ml/kg). Rotarod, Fast Trac running wheels and Catwalk (Noldus) gait analysis were used to assess motor performance, and neurological scoring was used to assess symptom onset. Mice were humanely killed at 90 days of age and tissue was fixed for histology or frozen.

Findings: We have adapted our human on human iMotorNeuron and iAstrocyte co-culture assay for high-throughput screening of small molecules. Following initial screening of candidate compounds identified by BenevolentAI, one promising hit (compound A) showed a robust and consistent motor neuron rescue in our co-culture model using C9orf72-ALS, SOD1-ALS and sporadic-ALS patient-derived iAstrocyte lines. Compound A inhibits several kinases, hence, we performed a secondary screen in the co-culture model, and identified four more specific kinase inhibitors that also provide a rescue effect, thus narrowing down the mode of action of compound A. In addition, compound A delayed neurological symptom onset from 69.3 to 74.1 days (p = 0.0447; one-way ANOVA) in the SOD1-G93A mouse model, suggesting potential therapeutic efficacy.

Conclusion: The AI technology developed by BenevolentAI has identified promising previously unknown targets for therapeutic approaches in ALS.

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C30 Meta-analysis of pharmacogenetic interactions in amyotrophic lateral sclerosis clinical trials

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Keywords: clinical trials, pharmacogenetic interactions

Objective: Disease heterogeneity in amyotrophic lateral sclerosis (ALS) complicates the development of effective treatment. We assessed whether genetic subgroups in recent trials responded to treatment with lithium carbonate, but noted that the treatment effect was lost in a large cohort of non-responders.

Methods: Individual participant data were obtained from three randomized trials investigating the efficacy of lithium carbonate. We matched clinical data with data regarding the UNC13A and C9orf72 genotype. Our primary outcome was survival at 12 months. On an exploratory basis, we assessed whether the effect of lithium depended on the genotype.

Results: Clinical data were available for 518 of the 606 participants. Overall, treatment with lithium carbonate did not improve 12-month survival (HR 1.0, 95% CI 0.7–1.4; p = 0.96). Both the UNC13A and C9orf72 genotype were independent predictors of survival (HR 2.4, 95% CI 1.3–4.3; p = 0.006 and HR 2.5, 95% CI 1.1–5.2; p = 0.032, respectively). The effect of lithium was significantly different for UNC13A carriers (p = 0.027), but not
for C9orf72 carriers ($p = 0.22$). The 12-month survival probability for *UNC13A* carriers treated with lithium carbonate improved from 40.1% (95% CI 23.2% – 69.1%) to 69.7% (95% CI 50.4% – 96.3%).

**Conclusions:** This is the first ALS study incorporating genetic data to determine treatment effects in a genetic post-hoc analysis. Our results suggest that we should reorient our strategies towards finding treatments for ALS and standardize the use of genotypic stratification in ALS clinical trials in order to optimize randomization and analysis for future clinical trials.

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Session 5B Pre-Approval Access

C31 Compassionate use of unapproved medicines: law, ethics, and policy

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Keywords: ethics, policy, expanded access

Pushed by patients and their advocates, countries are examining their policies concerning non-trial access to unapproved medicines, also known as ‘compassionate use’. Bateman-House, the co-chair of the NYU Working Group on Compassionate Use and Pre-Approval Access, will review how compassionate use in the United States has evolved over the last two decades and, in particular, the past 3 years. From the courtroom to the statehouse, from the FDA to Congress, there have been recent changes that are relevant to patients who seek access to investigational drugs; patient advocacy groups who seek to assist these patients; and the physicians who must guide these patients’ efforts to obtain unapproved drugs. Bateman-House will speak about the questions and concerns that pharmaceutical companies grapple with when deciding whether to make their unapproved medical products available for use via compassionate use. In addition to this legislative and policy update, she will offer suggestions for clinicians about ethical and pragmatic factors to be considered when dealing with a patient who would like to be treated with an unapproved medical product.

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C31A Improving access to investigational treatment: it’s not about the FDA

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Keywords: expanded access, adaptive trial design, real world evidence

Question: How can we make it easier for ALS/MND patients and their doctors to explore investigational medicines? Answer: By making meaningfully-sized Expanded Access Programs (EAPs) easier for drug companies to deliver. This presentation will (1) explain the fundamental factors of pre-approval access; (2) highlight the common misunderstandings of these factors in the context of ‘Right to Try’ narratives; and (3) present ways in which EAPs may benefit all parties in the drug development process.

First, to understand pre-approval access, we need to clarify what channels already exist. Since 1987, FDA regulations have permitted ‘treatment use clinical trials’ for patients who could not take part in research clinical trials. In the U.S., treatment use trials are regulated under 21 CFR 312 Subpart I, officially titled ‘Expanded Access’. Most developed nations have similar provisions that go by different names. U.S. regulations permit large-group ‘treatment use clinical trials’, aka Expanded Access Programs, for classes of indicated patients (ALS two years post-diagnosis, for example) who are not candidates for research trials. Alternatively, exceptional cases not belonging to any group can be handled through single-patient ‘compassionate use’ which, unlike classic Expanded Access, are sponsored on a case-by-case basis and accommodate small handfuls of patients.

Secondly, federal ‘Right to Try’ seeks to remove FDA oversight from this process. Thus, the ‘Right to Try’ concept should never be mistaken for existing practices of pre-approval access. Would sidestepping FDA make access programs more abundant? Or, on the other hand, could ‘Right to Try’ legislation serve only to displace more impactful initiatives and lower the likelihood of pre-approval access for most patients? Advocates and other stakeholders should consider the possibility that ‘Right to Try’ is the wrong approach to the right goal.

Thirdly, the biggest hurdle for early access is not the FDA. It is the commercial feasibility of running an access program, irrespective of the fate of ‘Right to Try’. We will review the costs and perceived hazards and discuss modern approaches for lessening the burden on drug companies. We will also examine ways in which access programs are being integrated into the drug development process, engaging wider, more representative ranges of patients within the indicated population, generating real world data, and informing the design of further research trials.

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Three hundred years ago, not far from the site of this year’s symposium, one of the most infamous events in American history occurred. A large number of young people were tried, convicted, and ultimately executed for witchcraft. The evidence in these ‘Salem Witch Trials’, which included ‘spectra’, ‘witch cakes’ and the ‘touch test’, was eventually debunked. The executions were recognized as a misunderstanding, the victims posthumously declared innocent, and their families compensated.

Today, many ALS neurologists seem to be on a witch hunt against the ‘Right to Try (RTT)’ law, and, more importantly, the very concept of patient self-experimentation.

I will show that our views on the RTT law probably don’t matter much; it will likely eventually be passed in most states and federally, and it is unlikely to result in any meaningful change. On the other hand, I will explain why I believe our views on the concept of patient self-experimentation are problematic. There are several more feasible pathways for patient self-experimentation, and surveys show that most patients with ALS are using at least one of these at some point in their illness. Also, there is evidence that patients want their doctors’ input. Against this background, I will review some of the arguments ALS neurologists make against patient self-experimentation, including the desire to ‘do no harm’, unfavorable risk benefit ratios, and fears about patient vulnerability, malpractice liability, extra work, and harming research studies. I will put each of these arguments ‘on trial’ and show that some are almost as flawed as the ones used in Salem.

By separating myths from realities, this talk should improve neurologists’ knowledge on patient self-experimentation and some common misconceptions they might have about it. In doing so, I hope it will facilitate shared-decision making.

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Session 6A SOD1 ALS: From Pathology to Therapy

C33 The familial G93A SOD1 mutation alters intrinsic electrical properties and morphological development of cortical interneurons

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Keywords: interneurons, in vitro, SOD1

Background: Mutations that affect interneuron function can produce devastating effects on neuronal circuitry and activity during development and adulthood. In the transgenic G93A SOD1 mouse model of ALS, previous research has identified that the disease course is marked by progressive and dynamic cortical interneuron involvement. However, it remains unclear if interneuron alterations represent a primary or secondary disease mechanism, and whether the mutation may prime interneurons to fail from early development, similar to cortical pyramidal neuron and motor neuron populations with this mutation.

Objective: To determine if cortical interneurons are innately susceptible to the G93A SOD1 mutation during development in primary cortical culture.

Methods: G93A SOD1 mice were crossed with the Gad67-GFP to ensure visualisation of cortical interneurons, which expressed the green fluorescent protein under the interneuron-specific glutamate decarboxylase 67 (Gad67) promoter. Primary mouse cortical cultures were derived from genetically isolated E15.5 Gad67-GFP:G93A SOD1 and Gad67-GFP:G93A SOD1 (wild-type) embryos. At 12 days in vitro whole-cell patch-clamp recordings were utilised for electrophysiological assessment of interneurons and cell morphology assessed from neurobiotin-filled processes that were post-hoc labelled with streptavidin and reconstructed using cell tracing software.

Results: We found that mutant G93A SOD1 significantly altered intrinsic firing properties and neuronal morphology of cortical interneurons. We also found a differential vulnerability of bipolar versus multipolar interneurons to disease. The divergent effect of mutant SOD1 in bipolar and multipolar interneurons notably affected outward potassium currents, which were significantly increased in bipolar interneurons (n = 6, G93A SOD1, 1.4 ± 0.13 nA; n = 5, C57Bl6, 0.84 ± 0.07 nA; p<0.05) and decreased in multipolar interneurons (n = 10, G93A SOD1, 1.1 ± 0.2 nA; n = 13, C57Bl6, 2.0 ± 0.2 nA; p<0.05). In addition, the neurite morphology of bipolar interneurons was unaltered while multipolar interneurons had significantly increased neurite complexity observed as increased branch number (n = 6, G93A SOD1, 135 ± 13; n = 9, WT, 98 ± 10, p<0.05) and neurite tree path length (n = 6, G93A SOD1, 15 ± 1.6 mm; n = 9, WT, 11 ± 0.7 mm, p<0.05). Furthermore, the duration of the action potential waveform was significantly decreased only in G93A SOD1 bipolar interneurons (n = 6, G93A SOD1, 10.48 ± 0.69 ms; n = 5, C57Bl6, 4.72 ± 0.99 ms; p<0.05), while current clamp recordings indicate G93A SOD1 decreased the intrinsic excitability of both multipolar interneurons (~50%) and bipolar interneurons (~30%) (p<0.05).

Discussion and conclusions: Our results have shown for the first time that cortical interneurons are innately vulnerable to the human G93A SOD1 mutation and suggest that differential priming of interneurons may be an early step in the initiation of disease.

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C34 Misfolded SOD1 pathology in sporadic ALS

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Keywords: SOD1, misfolded protein, immunohistochemistry

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by selective death of motor neurons in the cerebral cortex, brain stem, and spinal cord. ALS leads to progressive muscle weakness, atrophy and death usually 3 to 5 years after the onset. 10% of all ALS cases have affected relatives, known as familial ALS (FALS). The other 90% of cases are believed to be sporadic (SALS) in absence of familial history and known ALS-linked genetic DNA mutations. Cytoplasmic mutant SOD1-positive aggregates, in both SOD1-linked patients and transgenic mouse models overexpressing different FALS-associated human SOD1 mutants, have been extensively described. It has been also interestingly reported that wild-type (WT) SOD1 can acquire aberrant misfolded conformation, implying a shared pathological pathway between SALS and SOD1-linked FALS, and exacerbate disease presentation in FALS-SOD1 mouse models.
To answer the controversial question whether or not misfolded SOD1 (misSOD1) accumulation is a common pathological feature in SALS, we have undertaken an unbiased blinded histological and biochemical analysis of misSOD1 accumulation in different post-mortem CNS tissues (brain and spinal cord) from 18 SALS and 1 FALS patients, as well as 2 non-neurological controls. 5 different monoclonal antibodies, raised by different independent investigators and previously proven to be highly specific for human misSOD1, with epitope encompassing the entire SOD1 protein were used throughout this study. The experiments were performed using an automated tissue and slide-staining device. This unbiased, blinded, standardized and automated approach provides strong evidence that misSOD1 is a principal component of sporadic ALS as misSOD1 staining (aggregates and diffuse) was detected in both post-mortem brain and spinal cord tissue sections from all of the tested SALS cases.

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C35 Distinct neuronal inclusions containing misfolded SOD1 in patients with mutations in C9orf72 and other ALS- and FTD-associated genes

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Keywords: SOD1 C9orf72 neurons

Background: A common cause of amyotrophic lateral sclerosis (ALS) is mutations in the superoxide dismutase-1 (SOD1) gene (1). The main pathological hallmarks of this type of ALS are inclusions containing mutant SOD1 in motor neurons and glial cells (2). We have earlier reported the finding of small, granular intraneuronal inclusions of wild-type SOD1 in motor neurons of the spinal cord in ALS patients that do not carry SOD1 mutations (3).

Objective: To search for SOD1 inclusions in autopsied patients carrying different ALS causing genes.

Methods: Tissues were collected at autopsy at Umeå University Hospital, Sweden and at Danish Pathology units from patients with ALS, FTD, or ALS-FTD, diagnosed clinically in accordance with the EFNS Consensus Criteria for Managing ALS and according to the Neary Criteria for FTD. A total of 18 patients carrying a repeat-expansion in C9orf72, four patients with mutations in FUS, Alsin and VAPB and 17 patients with SOD1 mutations were included. Also 10 control patients with other neurodegenerative disorders as well as 20 non-neurological control patients were included. Using a panel of antibodies that specifically recognize misfolded SOD1 species, brain and spinal cord sections from these patients were immunohistochemically stained and analysed.

Results: All 18 patients examined with a repeat expansion in C9orf72 had distinct inclusions of misfolded SOD1 in motor neurons of the spinal cord. Similar inclusions were occasionally also found in the medulla oblongata and in neurons of the motor cortex and frontal lobe. Four patients with mutations in FUS, Alsin, and VAPB, carried similar misfolded SOD1 inclusions. No inclusions were observed in 20 patients who had died from non-neurological causes and they were detected in small amounts in only 1 of 10 patients with other neurodegenerative diseases. Comparison was made with 17 patients with nine different SOD1 mutations. Morphologically, the inclusions in patients with mutations in C9orf72, FUS, VAPB, and Alsin resembled inclusions in patients carrying the wild-type-like SOD1 mutation D90A whereas patients carrying unstable SOD1 mutations (ie A4V, D83G, V5M, G114A and G127X) had larger skein-like SOD1-positive inclusions.

Discussion: These results indicate that the morphology of the inclusions could be related to the stability of the SOD1 protein. The finding that distinct inclusions containing misfolded SOD1 are found in patients carrying mutations in other genes predisposing to ALS and FTD suggests that misfolding of wild-type SOD1 can be part of down-stream pathogenic events.

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C36 Development of peptides that specifically recognize misfolded SOD1 proteins in amyotrophic lateral sclerosis

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C37 Peptide-directed selective knockdown of misfolded SOD1 as a therapy for ALS

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Keywords: SOD1, protein-knockdown, autophagy

Background: Over the past two decades, a major breakthrough in ALS research is the discovery that mutations in the gene Cu/Zn superoxide dismutase type1 (SOD1) are a cause of familial ALS. Familial ALS (FALS)-linked SOD1 variants are readily susceptible to post-translational modifications, and subsequently become misfolded. Failure in degradation of unfavorable SOD1 with ubiquitin and/or autophagy proteasome system leads to accumulation of SOD1 aggregates and triggers a toxic cascade leading to motor neuron degeneration. Notably, wild-type SOD1, when modified post-translationally, undergoes aberrant conformational changes and acquires the same toxic properties as FALS associated SOD1 variants. Thus, misfolded SOD1 is a common toxic factor to a subset of both familial and sporadic ALS. Selective clearance of misfolded SOD1 is therefore a rational approach towards developing effective therapies for ALS.

Methods: Here we report a peptide-direct protein knockdown system that rapidly and selectively degrades misfolded SOD1 through a chaperone-mediated autophagy pathway. The peptide system consists of a cell membrane-penetrating domain that allows the peptide to bypass the blood-brain barrier and plasma membrane following peripheral delivery, a cytosolic carboxyl terminal region of the Derlin1 CT4 motif that selectively binds to misfolded SOD1, and a chaperone-mediated autophagy targeting motif (CTM) that directs the peptide-protein complex for lysosomal degradation.

Results: Co-expression of the peptide with either the G93A or the G37R mutations of human SOD1 shows a significant reduction of the levels of both G93A and G37R, but not the wild-type SOD1 in the control group. Inducing misfolding of human SOD1 by serum deprivation or increasing lysosomal activity robustly increases the knockdown efficiency of misfolded SOD1. The knocking down function of the peptide can be completely abolished when either the CT4 domain or the CTM domain is mutated.

Discussion: Our in vitro data so far show that the peptide system effectively knocks down misfolded SOD1 in a dose, time and lysosomal activity-dependent manner. Intravenous injection of a single dose (10 mg/kg) of the 37-aa synthetic peptide in the G37R and the G93A mice resulted in a 57% reduction of misfolded SOD1 in 24 h. We are currently testing the effectiveness of the peptide in animal models of ALS, and are hopeful that the peptide-direct protein knockdown system can be potentially developed into a cure for ALS.
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C38 A promising small molecule lead in the search for a SOD1-targeted drug for ALS

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Keywords: SOD1, drug development, structural function

Background: SOD1 is an important part of our defence against oxidative stress but it is also a cause of amyotrophic lateral sclerosis. Reduced ability to metalate, dimerize and form an intra-subunit disulphide bond are characteristic of ALS-mutant SOD1. This facilitates formation of toxic oligomers and aggregates, ultimately leading to neuronal death.

Methods: Using an information rich combination of crystallographic ligand determination, native mass spectrometry, in-cell and in vitro NMR we investigated the bi-modal action of an anti-oxidant compound on pathogenic mutant SOD1 behaviour. This compound exhibits a SOD1 pharmacological chaperone role that is determined by covalent addition at two sites.

Results: We found that it forms a bond with SOD1 at Cys111 in vitro and restores the monomer-dimer equilibrium of A4V SOD1 to wild-type levels. It does this without cross-linking opposing monomers and does not hinder heterodimerization with hCCS. In the cytosolic environment it is exceedingly efficient at directing correct SOD1 folding and normal post-translational modification events. This almost completely depopulates the globally unfolded potentially toxic precursor which predominates when mutant SOD1 is expressed in cells.

Discussion: This compound’s structure, history, previous usage and broad anti-oxidant properties draw comparisons with edaravone. It is well tolerated in humans and penetrates the blood-brain barrier. Its secondary pharmacology together with its primary SOD1 pharmacological chaperone role indicates it is a very promising lead molecule in SOD1-ALS therapeutics development. Experiments are currently underway in transgenic mice.

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C39 Characterization of ALS patients based on MIP and FVC at first visit

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Keywords: maximal inspiratory pressure, forced vital capacity, ALSFRS

Background: According to AAN practice parameters, initiation of NIV is indicated with a forced vital capacity (FVC) ≤ 50% or when maximal inspiratory pressure (MIP) ≥ 60 cmH2O (1). Studies have shown that MIP is a more sensitive indicator of hypoventilation compared to FVC (2). We hope to replicate these findings and further characterize ALS patients based on MIP and FVC measured at first visit.

Methods: This is a retrospective database cohort study of 264 ALS patients from 2014 to 2017. Demographic variables were analyzed using the Kruskal-Wallis test for nominal and Fischer’s exact test for categorical variables. The patients were categorized based on FVC and MIP at first visit: (G1: FVC < 50%, MIP < 60; G2: FVC ≥ 50%, MIP ≤ 60; G3: FVC < 50%, MIP ≤ 60) and compared based on disease variables. Multi-visit analysis (≥ 2 visits and ≥ 6 months) was conducted for time to MIP ≥ 60 and FVC ≤ 50 for 158 patients not meeting criteria for NIV initiation at time of diagnosis. Statistical significance was set at p < 0.05.

Results: 264 ALS patients were analyzed (61.4 years ± 11.9; 40% F; 21.2% bulbar; 18.7 months ± 15.2 disease duration; 81.2% on riluzole). At first visit, 8.7% met NIV criteria by FVC ≤ 50 vs. 58.33% for MIP ≥ 60. For all other patients (n = 158), 74 reached FVC ≤ 50% at 11.8 months ± 9.1 vs. 126 reaching MIP ≤ 60 at 3.83 months ± 7.3, with FVC lagging by 7.94 ± 7.8 months to reach criteria. For patients grouped according to FVC and MIP at diagnosis (G1, G2, G3), a greater proportion of patients with older age, female gender, bulbar status, faster pre-diagnosis progression rate was noted in groups II and III (MIP ≤ 60; p < 0.0005).

Conclusions: As previously published, MIP is an early, sensitive indicator for initiation of NIV. In addition, older female patients with greater proportion of bulbar disease and faster pre-diagnosis progression rate, are likely to present at time of diagnosis meeting criteria for NIV by MIP, independent of FVC. Additional information will be presented on impact of NIV on rate of progression in these patients. Prospective studies are needed to better characterize and correlate these parameters to treatment indication and outcomes.

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C40 Comparison between slow and forced vital capacities on survival prediction in amyotrophic lateral sclerosis

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Keywords: slow vital capacity, forced vital capacity, survival

Introduction: Slow (SVC) and forced (FVC) vital capacities are the most used pulmonary function tests in amyotrophic lateral sclerosis (ALS). It is unknown if they equally predict survival in ALS. The aim of the present study was to compare both measures in predicting survival in this disease.

Methods: Consecutive definite/probable ALS patients (2000–2014) in whom respiratory tests were performed at baseline and four months later were included. All were evaluated with the revised ALS functional rating scale (ALSFRS-R); respiratory (RofALSFRS-R), bulbar (ALSFRSb), upper and lower limb subscores; SVC; FVC; maximal inspiratory (MIP); expiratory (MEP) pressures. King’s functional staging system was applied retrospectively. Survival analysis was carried out by univariate Kaplan-Meier log-rank test. Multivariate Cox proportional hazards model determined significant independent variables.
Results: We included 469 patients (270 males; mean onset age 61.0 ± 11.5 years; mean disease duration from first symptoms to first visit: 15.8 ± 16.1 months; 329 spinal and 140 bulbar onset). FVC and SVC were strongly correlated ($r^2 = 0.981, p < 0.001$). Significant survival prognostic variables obtained from the Kaplan-Meier analyses were onset region, onset age, disease duration, ALSFRS-R, ALSFRSb, RoALSFRS-R, ALSFRS-R decay, SVC, FVC, MIP, MEP, King’s staging ($p < 0.01$). Final Cox model including the significant variables showed similar results for FVC and SVC ($p < 0.001$). Moreover, 1% decrease in either predicted values increased death probability by 1.02.

Conclusion: FVC and SVC are strongly correlated and are inter-changeable in predicting survival in ALS.

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C41 How good is the respiratory subscore of ALSFRS-R?

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Keywords: ALSFRS-R, forced vital capacity, non-invasive ventilation

Background: Respiratory assessment is of primary prognostic importance in ALS. The respiratory subscale of the revised ALS functional rating scale (ALSFRS-R) is an attempt to quantify respiratory dysfunction from symptoms. Metric qualities and reliability of the subscore as a prognostic marker have been questioned.

Objectives: To examine the sensitivity and reliability of the respiratory subscore of ALSFRS-R (RespSS) and its constituent questions as measures of respiratory dysfunction in ALS. Additionally, to explore utilization of non-invasive ventilation (NIV) by examining responses to question 12 of ALSFRS-R.

Methods: Forced vital capacity (expressed as percent of normal, or FVC%) and ALSFRS-R (including RespSS) measures obtained on the same day were extracted from the PRO-ACT dataset. FVC% was taken as the objective criterion of respiratory dysfunction. Logistic regression and mixed-effects models were constructed to examine the relationship of RespSS questions and score to FVC, and identify predictors.

Results: At least 1 same-day measurement of FVC% and ALSFRS-R was obtained in 1874 patients. A total of 8742 measurements were obtained. Median initial FVC% was 85%, with interquartile range (IQR) 75–96%. Median initial RespSS was 12 (normal), with IQR 11–12. Mean linear rates of decline from initial measurement were −2.65% per month for FVC% and −0.17 points per month for RespSS. If measured from onset of weakness, RespSS had a slightly concave or accelerated trajectory. Steeper initial ALSFRS-R slope, bulbar onset and older age were independent predictors of steeper FVC% decline. Steeper initial ALSFRS-R slope, male gender and older age were independent predictors of steeper RespSS decline. RespSS and FVC% were moderately correlated ($r = 0.46$), but their relationship was curvilinear. Decline registered on RespSS only after FVC% dropped below 80% and the RespSS declined progressively more rapidly with further decline in FVC%. Question 10 of ALSFRS-R (about dyspnea) was the most sensitive, whereas question 12 (about NIV) was the least sensitive. Adjusted for FVC%, age, bulbar onset, and initial ALSFRS-R slope, there was greater inter-patient variability with question 12 than with questions 10 and 11, and females were less likely to score on question 12. Of 935 instances when FVC% was 50% or less, NIV was employed only in 487 (52%). Of 250 instances when FVC% was 30% or less, NIV was not employed in 84 (34%).

Conclusion: RespSS is an insensitive and unreliable marker of early respiratory dysfunction in ALS. Specifically, responses to question 12 relating to NIV are largely determined by patient preferences. Even in the PRO-ACT cohort that presumably received optimal care, NIV seems to have been under-utilized, especially by females. RespSS may benefit from revision.

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C42 Cough assist: using the flow and pressure graphics to improve patient outcomes

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Keywords: cough assist, lung volume recruitment, airway collapse

Background: Graphic Analysis of Pressure, Flow and Volume are used to improve patient ventilator synchrony during mechanical ventilation (1). However, real-time graphic displays are not similarly available on cough assist (CA) devices for use in improving Peak Cough Flow (PCF). Graphic analysis on the CA device requires downloading the data to the secure digital (SD) card, uploading to a computer and then viewing the waveforms using DirectView software.

Objectives: The primary objective of the study was to determine how graphic waveforms might be used to customize settings for individual patients with the intent of improving patient outcomes. A secondary objective was to evaluate how the device might be used to support Lung Volume Recruitment (LVR) using Long, Slow, Deep technique with a target pressure of 40 cmH2O (critical opening pressure).

Methods: Data cards were collected over a 6-month period from patients receiving CA support and referred to Respiratory Quality Services (RQS) for monitoring and follow-up. The graphics data from the CA devices were used to evaluate the effectiveness of the CA settings
including inspiratory and expiratory pressure, inspiratory flow, and both inspiratory and expiratory time. The effectiveness of the patient cough technique and timing with the onset of negative pressure were evaluated. Graphics analysis was also used to analyze the effectiveness of the device when used for LVR.

**Results:** The graphics analysis resulted in recommendations for changing at least one CA setting on all patients \((n = 34)\). The two primary changes were: increasing the inspiratory and expiratory pressure settings to improve PCF; and adjusting the inspiratory time-setting based on the pressure control algorithm so that the patient cough effort could be synchronized with the onset of the negative pressure. Changes in pressure settings resulted in a mean increase in PCF of 32%. Preliminary results from 6 bulbar patients indicate that graphic analysis can be used as a non-invasive method for titrating negative pressure to prevent upper airway collapse.

**Discussion and conclusions:** Prior to this, our impression was that the vast majority of CA settings have been made without any objective method for evaluating efficacy. A simple stepwise analysis using the flow and pressure graphics supports individualizing the settings specific to each patient and improves peak cough flow. Graphics analysis has also led us to using the CA device as a lung volume recruitment tool, where breaths are delivered using a target pressure of 40 cmH\(_2\)O or higher and a five second inspiratory time (Long, Slow, Deep).

**C43 Mechanical insufflation exsufflation and lung volume recruitment in amyotrophic lateral sclerosis: a prospective study of the prescription process, the outcomes and the experience**

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**C43 Mechanical insufflation exsufflation and lung volume recruitment in amyotrophic lateral sclerosis: a prospective study of the prescription process, the outcomes and the experience**

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**Keywords:** mechanical insufflation exsufflation, lung volume recruitment

**Background:** Mechanical Insufflation Exsufflation (M-IE) and Lung Volume Recruitment (LVR) are two airway clearance devices that augment cough strength in amyotrophic lateral sclerosis (ALS) \((1)\). Changes in respiratory measures, morbidity, physical function and outcomes following prescription of M-IE and LVR require investigation. The characteristics of participants prescribed M-IE and LVR devices and their experiences with these devices have not been reported.

**Objectives:** The primary aim was to measure respiratory muscle function, cough strength, chest infection morbidity, physical function and outcome in an Irish ALS cohort over one year. The second aim was to examine prescription practices in relation to M-IE and LVR and to compare participant characteristics in those prescribed these devices with those who were not. The third aim was to evaluate the experiences of participants who were prescribed M-IE and LVR.

**Methods:** A prospective longitudinal study assessed 108 participants at several time-points over one year. Respiratory measures, including Sniff Nasal Inspiratory Pressure (SNIP), Slow Vital Capacity (SVC) percent predicted, Peak Cough Flow (PCF), physical function measured using the Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) and outcomes including chest infection rate and mortality were measured. Participant characteristics were evaluated at device prescription and a 13 item self-administered questionnaire evaluated their experience and usability with M-IE and LVR.

**Results:** Participants including 85 spinal-onset and 23 bulbar-onset patients, of mean age 62.05 ± 11.47 years were recruited. All measures including SNIP, SVC percent predicted, PCF and ALSFRS-R declined significantly \((p<0.001)\) at a rate of 18.72 cmH\(_2\)O, 17.49%, 124.84 L/min and 9.62 units per year, respectively. One-third reported a chest infection and 19.44% died. The rate of prescription of a device during the 1-year study period was 29.63% \((32/108)\). Slightly more LVR devices \((18, 56.3\%)\) than M-IE devices \((14, 43.8\%)\) were prescribed. Participants prescribed a device had significantly lower average SNIP, SVC percent predicted, PCF and ALSFRS-R \((p<0.001)\). Site of onset and time from diagnosis was not significantly different to participants not prescribed a device. Results of the questionnaire indicated that M-IE and LVR were user friendly; with a high level of adherence reported. Additionally, M-IE and LVR increased self-reported cough strength, although there was a low level of agreement that the devices increased speech volume and the self-reported increase in cough strength during a chest infection was undetermined.

**Conclusion:** The significant rate of decline of SNIP, PCF, SVC percent predicted and ALSFRS-R is consistent with previous reports. M-IE and LVR were frequently prescribed and participants prescribed these devices had lower respiratory function and ALSFRS-R scores. ALS participants report a positive experience with M-IE and LVR use and M-IE and LVR are user friendly.

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C44 A US randomized trial of DPS in ALS: the outcome differs from two European trials

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Keywords: diaphragm pacing system, hypoventilation, clinical trial

Objective: To determine if diaphragm pacing improves survival in ALS patients with hypoventilation.

Background: DPS was originally approved for the treatment of hypoventilation in ALS, based on early work suggesting subjects using DPS and NIV had improved survival compared to historical controls using NIV alone. Subsequently, we planned a randomized trial because it was never clear that these controls were identical to patients who underwent DPS and the reports were potentially subject to biases. During the course of our trial, two European controlled trials on DPS reported that DPS was associated with reduced survival and, as a result, we stopped enrolling patients before completion, but continued observing those patients who had already been implanted.

Methods: This was an unblinded, randomized trial comparing DPS to standard of care (SOC). Subjects had FVC less than 50% predicted or MIP less than 60 with recordable bilateral hemidiaphragms by phrenic nerve stimulation. They were recruited from 20 US ALS centers and were randomized 2:1 to either laparoscopic DPS surgery or SOC. The primary outcome was survival, analyzed both in the intention-to-treat population, and in the surgical versus non-surgical population. Treated patients were followed for at least 18 months. Safety outcomes were also assessed.

Results: We aimed to enroll 180 patients, but enrolment was initially slow, and slowed further after the 2 European studies found that DPS might be harmful. As a result, enrolment ceased after 52 patients. In our intent to treat group (ITT), 32 subjects were randomized to surgery and 20 to SOC. Our surgical treatment group ultimately included 22 DPS patients with 30 receiving standard of care. There were 28 total deaths. The hazard ratio was 0.746 in favor of DPS. 90% confidence intervals were 0.39 to 1.41. The estimated median survival was 19 months for DPS and 17 months in SOC. By surgical treatment the naïve estimate of the hazard ratio was 0.82, with confidence intervals of 0.44 to 1.54. The estimated median survivals were 19 months in the DPS group and 17 in SOC.

Conclusion: We found no significant differences between DPS and SOC in ALS, using either analysis, with a mild advantage favoring DPS. These results clearly differ from the two earlier European trials. Our median survival of 19 months was similar to the the 19-month median survival observed in the earlier open label study of DPS, but was far better than the 11-month survival reported in the study from England. In our analysis, the different trial conclusions relate to varied methodologies, treatments and chance: specifically, the way NIV was used in the British study and the strikingly positive survival in the control group from the French study. These small studies leave open questions about the utility of DPS.

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Session 6C Biofluid Markers

C45 Urinary p75 neurotrophin receptor extracellular domain: a biomarker relevant to ALS therapy development

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Keywords: progression biomarker, urinary p75ECD, pharmacodynamic biomarker

Background: There is an urgent need for validated diagnostic, prognostic, disease progression and pharmacodynamic biomarkers that might aid ALS therapy development (1). The paucity of such biological fluid-based biomarkers has led us to focus on the common neurotrophin receptor (p75) extracellular domain as a biomarker of motor neuron degeneration.

Objective: To evaluate urinary neurotrophin receptor p75 extracellular domain (p75ECD) levels as an ALS biomarker with potential application specifically to predicting prognosis; quantifying disease progression and potential pharmacodynamic effect; and potentially quantifying pre-symptomatic disease in healthy individuals at genetic risk for developing ALS (psALS).

Methods: This study involved 45 healthy controls and 54 people with ALS (2), 31 of whom were sampled 2–6 times over a 2-year period, which confirmed our previous results (3). In addition, we examined 68 psALS, 10 of whom have phenocverted during longitudinal follow-up, with samples from before and after diagnosis available in 5f. Urinary p75ECD was measured using an enzyme-linked immunosassay and validation included intra- and inter-assay coefficients of variation; effect of circadian rhythm; and stability and repeated freeze-thaw cycles. Longitudinal changes in urinary p75ECD were examined by mixed model analysis, and the prognostic value of baseline p75ECD explored by survival analysis.

Results: Assay reproducibility is high, with p75ECD showing stability across repeated freeze-thaw cycles, at room temperature and 4°C, with no diurnal variation. Urinary p75ECD is higher in ALS patients compared to controls (p<0.0001), and correlates with ALSFRS-R at baseline (r = -0.44, p = 0.008) and across all study visits (r = -0.36, p<0.0001). p75ECD increased as disease progressed at an average rate of 0.19 ng/mg creatinine per month (p<0.0001). In multivariate prognostic analysis, bulbar onset (hazard ratio (HR) = 3.0, p = 0.0035), faster rate of ΔFRS (HR = 4.4, p<0.0001), and higher baseline p75ECD (HR = 1.3, p = 0.0004) predict worse survival. Analysis of p75ECD in the psALS population is ongoing with results to be included in final presentation.

Conclusion: The assay for urinary p75ECD is analytically robust and promising as an ALS biomarker with prognostic, disease progression, and potential pharmacodynamic application. Urinary p75ECD is currently the only biological-fluid-based biomarker of disease progression, and has potential for use in ALS clinical trials. Ongoing studies will shed light on when in the natural history of ALS p75ECD first begins to increase and what potential it might have for predicting the onset of disease.

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C46 Blood and CSF neurofilament levels as biomarkers of pre-symptomatic disease

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Keywords: pre-symptomatic, biomarker, disease onset

Background: Neurofilaments are cytoskeletal intermediate filaments specific to neurons and axons, and have been extensively studied as potential biomarkers in neurological disorders. Several studies have shown that, in cerebrospinal fluid (CSF) and blood, levels of neurofilament light (NFL) and phosphorylated neurofilament heavy (pNfH) are elevated in ALS patients compared to healthy controls. Moreover, levels of NFL and pNfH remain largely stable over time as ALS progresses. Hitherto unknown is when in the natural history of ALS, levels of NFL and pNfH begin to increase. Prior efforts to address this question have been limited by the absence of data from ALS-causing gene mutations carriers followed longitudinally from the pre-symptomatic state through phenoconversion (the development of clinically manifest disease).

Objectives: To identify biochemical biomarkers of pre-symptomatic disease in clinically unaffected individuals at
C47 CSF pNfH as a diagnostic and prognostic biomarker in ALS: experience with a colorimetric sandwich immunoassay

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**Methods**: The Pre-Symptomatic Familial ALS (Pre-FALS) study is a longitudinal natural history and biomarker study of pre-symptomatic individuals who are carriers of an ALS-associated gene mutation. NfL in blood and CSF have been measured, using a published immunoassay utilizing a selection of monoclonal and polyclonal antibodies, in 56 samples from 34 controls/gene mutation non-carriers; 46 samples from 19 affected individuals; 214 samples from 84 Pre-FALS gene mutation carriers who have not yet phenoconverted (‘pre-symptomatic’); and 30 samples from 11 Pre-FALS phenoconverters. Efforts to analyze pNfH and anti-neurofilament antibody levels are underway.

**Results**: In cross-sectional analysis, serum NfL detected by electrochemiluminescence immunoassay are significantly (p<0.001) elevated among ALS patients compared to controls and pre-symptomatic individuals. In longitudinal analyses, levels of NfL in controls and pre-symptomatic are generally stable over time and remain below a level of 55 pg/ml. By contrast, in the phenoconverter group, among the 8 with longitudinal samples from before and after phenoconversion, NfL levels begin to increase during the 12-month period preceding the appearance of clinically manifest disease. By the time of diagnosis, levels of NfL are always above 55 pg/ml and, as observed in the affected group, remain largely stable as disease progresses. There is a strong correlation (r = 0.9, p<0.001) between serum and CSF NfL with similar temporal patterns observed for CSF NfL.

**Discussion and conclusions**: Here we provide the first-ever insight into the longitudinal trajectory of blood and CSF NfL levels quantified in the same individuals both before and around the time of phenoconversion to clinically manifest disease. These data show that NfL levels rise within 12 months prior to phenoconversion, providing evidence for its potential both as a biomarker of pre-symptomatic disease and as a prognostic marker predicting the short-term likelihood of developing clinically manifest disease. These findings open up the possibility of an early therapeutic and/or disease prevention trial.

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**Keywords**: pNfH, biomarker, CSF

**C47 CSF pNfH as a diagnostic and prognostic biomarker in ALS: experience with a colorimetric sandwich immunoassay**

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**Background**: Multiple studies over more than 20 years have demonstrated increased neurofilament (NfH or NfL) levels in the CSF of patients with ALS. These studies have increasingly extended to consider NFs as a prognostic biomarker, and in the differentiation of ALS from mimic disorders rather than solely healthy age-matched controls.

**Methods**: We applied a CE-marked ELISA assay (Euroimmun, Germany) for CSF pNfH to the ‘BioMOx’ longitudinal cohort comprising ALS patients across a range of clinical phenotype (n = 76), healthy controls (n = 28) and a group of disease mimics seen in a tertiary ALS referral centre (n = 13). Each assay used 25 ml of CSF. We assessed first-visit pNfH in relation to absolute ALSFRS-R, its rate of change, disease duration from symptom onset and survival to census. Six-monthly longitudinal sample levels in the ALS group were also measured.

**Results**: CSF pNfH levels were higher in ALS patients versus healthy controls (p<0.0001) and versus disease mimics (p<0.0001). The inter-assay CVs were mostly under 10% and the mean intra-assay CVs all below 10%. Using receiver operating curve analysis, an optimal pNfH cut-off discriminated patients with ALS from healthy controls with high sensitivity of 91% (CI 82–96) and specificity of 100% (CI 88–100). pNfH retrospectively differentiated patients with ALS from disease mimics with a sensitivity of 91% (CI 82–96) and specificity of 85% (CI 55–98). A positive correlation was demonstrated between CSF pNfH and rate of decline of ALSFRS-R (r = 0.590), and an inverse correlation between CSF pNfH and disease duration (r = −0.548). Kaplan-Meier survival analysis showed a clear separation between sub-groups of patients binarized into high and low first-visit pNfH levels (p<0.001). Individual longitudinal data analysis will be presented.

**Conclusion**: A commercially available CSF pNfH assay validated for clinical diagnostics, applied to a retrospective cohort, has high diagnostic accuracy for ALS in a tertiary referral clinic setting. CSF pNfH levels are linked to survival, with ongoing potential as an early proof-of-principle or pharmacodynamic biomarker in future therapeutic trials.

**Disclosure**: ELISA kits were provided in-kind by Euroimmun. No payment was received for this study, and the analysis was carried out independently (EG).

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C48 Lipidomics reveals cerebrospinal fluid signatures of ALS

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Keywords: lipidomics, cerebrospinal fluid, phosphatidylcholine, ceramide

Objective: The objectives of this study were to investigate the cerebrospinal fluid (CSF) lipidomic signature of ALS patients to evaluate the diagnostic and predictive values of the profile and to identify pathophysiologic biomarkers.

Methods: We performed an untargeted lipidomic analysis in 40 ALS patients compared to 45 controls. We robustly determined 122 lipids by liquid chromatography coupled to high-resolution mass spectrometry. Parameters of disease progression were collected at baseline and again one year later (ALSFRS-R, FVC, BMI), as well as survival. Lipid profiles were then subjected to powerful statistical modelling to compare ALS and controls and to model the rate of progression.

Results: ALS displayed a highly significant specific CSF lipidomic signature involving phosphatidylcholines, sphingomyelins and triglycerides. Phosphatidylcholine PC(36:4), higher in ALS (p = 0.0003) was the strongest biomarker. Analysis of lipids in the brain cortex of ALS mice confirmed the role of some discriminant lipids such as PC. We also obtained an excellent model (accuracy of 79%) for predicting the variation of the ALSFRS-R score to model the rate of progression.

Interpretation: Our study, which shows extensive lipid remodelling in the CSF of ALS patients, provides new biomarkers of the disease and its evolution. Importantly, the lipidomic signature found in ALS patients is consistent with ALS mice findings, highlighting phosphatidylcholines that merit further exploration.

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C49 Unravelling phenotypic heterogeneity in ALS using quantitative proteomics: from animal models of the disease to human pathology

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Keywords: proteomics, biomarkers, plasma

Background: The poor record of therapeutic success in amyotrophic lateral sclerosis (ALS), depends on the late recognition of the disease process which narrows the window of therapeutic opportunities. An immunological response to the disease is known to occur systemically and in affected tissues in ALS, which is likely to be linked to the variability in progression rate and survival observed in affected individuals. While a modest response has been shown in ALS animal models to different treatments, the same therapeutics have failed to have a modifying effect of human pathology. This discrepancy hinges on a fundamentally diverse pathobiology of the disease across species and/or on the lack of a rigorous pre-clinical study design. To advance ALS therapeutics, we need a better insight into the main drivers of the biological and clinical variability of the disease. Understanding how ALS pathology compares in human and rodents will inform the best use of animal models as surrogates of human pathology.

Aims: To obtain a comprehensive coverage of the biological factors implicated in the phenotypic variability of ALS, with a focus on the inflammatory response, rate of progression and disease stage. The main objective is to compare protein signatures in plasma samples from ALS individuals with a fast and slow rate of disease progression, at an early and late stage of the disease. We also studied plasma/PBMC co-expressed proteins in fast (C57 background) versus slow (129Sv background) progressing G93A mouse models to describe an ontology of regulated biological factors implicated in the phenotypic variability of the disease. Understanding how ALS pathology varies across species and/or on the lack of a rigorous pre-clinical study design.

Methods: TMT labelling-based proteomic approach has been used to obtain the data presented in this study.

Results: The human proteome seemed to change more radically with the disease stage and less with rate of progression. Early regulated factors encompassed the innate immune response of MCH2, NK regulation, phagocytosis and apoptosis, while the later stage proteome included mostly regulated cytoskeletal and metabolic effectors involved in (RHO) GTPase signalling. The SOD1 mouse fast vs. slow plasma/PBMC proteome was substantially different and shared with the human some of the biological features linked to immune response and
metabolism. Trends of differential regulation of 4 different proteins was further tested using immune-detection methods in a different cohort.

**Discussion:** The enrichment in substantially different biological features at different time-points identified in our study may support therapies which should be tailored to different biological targets in the various phases of the disease. This study provides a framework to unravel the biological complexities of the animal surrogacy to patients. It also provides a formidable platform for the future validation of disease biomarkers in ALS bridging human and animal pathology.

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**C50 Longitudinal analysis of the CSF proteome in ALS: emerging microglial markers**

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**Keywords:** biomarkers, CSF, human

**Background:** The proteomic signature of cerebrospinal fluid (CSF) has obvious biomarker potential in ALS, but there have been few longitudinal analyses to date. We sought to perform state-of-the-art proteomic analysis of individual CSF samples from a well-characterised longitudinal cohort of ALS patients, healthy and disease controls.

**Methods:** CSF samples were available from the longitudinal ‘BioMOx’ cohort of 75 subjects with ALS and PLS (including up to five longitudinal time-points), 20 healthy controls, 10 subjects with ALS mimic disorders and 20 subjects with Parkinson’s disease. 50 mL CSF samples were digested using a bead immobilized heat stable trypsin prior to analysis. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was performed with label-free quantification of identified proteins.

**Results:** Over 762 proteins have been identified and quantified across the groups. Cross-sectional analysis demonstrated significant differences in the abundance of over 60 proteins (FDR <0.05) between ALS and other groups. There was consistent elevation of the macrophage enzymes chitotriosidase (CHIT1) in ALS compared with other groups. Interestingly, elevation of chitinase 3-like protein 1 (CHI3L1), was also observed in ALS compared with all groups except PLS, in which CHI3L1 was also elevated.

CHIT1 and CHI3L1 were positively correlated with disease progression rate (CHIT1 Pearson’s r = 0.58 p<0.001, CHI3L1 r = 0.41 p<0.001), while high CHI3L1 was associated with shortened survival from the time of sampling (p = 0.0091).

Additional data on longitudinal changes, survival analysis and receiver operating characteristic analysis will be presented along with gene ontology enrichment and pathway analysis.

**Conclusions:** We have implemented a high-throughput LC-MS/MS method for in-depth proteomic analysis of individual CSF samples in a longitudinal cohort. This has identified elevation of markers of microglial function in ALS, in agreement with evidence from disease models and imaging studies. As such they might represent promising markers of target engagement for candidate immunomodulatory ALS therapies. Validation is required to assess their usefulness in diagnosis and prognostication.

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Session 7A TDP-43

C51 Dynamic polymerization of TDP-43 in health and disease

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Keywords: TDP-43, splicing regulation, pathological aggregation

TDP-43 is a primarily nuclear RNA-binding protein (RBP), whose abnormal cytoplasmic accumulation characterizes affected neurons in patients with amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). TDP-43 is a nucleo-cytoplasmic shuttling protein comprising two RNA recognition motifs (RRMs) that bind TG-/UG-repeat nucleic acids in a sequence-specific fashion and are indispensable for its roles in RNA metabolism. TDP-43 also contains a C-terminal prion-like or low complexity domain (LCD), mediating protein-protein interactions and also its incorporation into stress granules, potentially via its property to phase separate. Furthermore, the TDP-43 LCD is crucially involved in disease, since it is proteolytically cleaved and abnormally phosphorylated, leading to its cytoplasmic accumulation in complex with the full-length protein. Finally, TDP-43 contains an N-terminal region spanning its first 80 amino acids, whose role in the function and/or malfunction of the protein remains unclear.

Due to the lack of sequence homology of this region with any known structures, the N-terminal domain (NTD) of TDP-43 was initially thought to be unstructured. However, it was recently reported that monomeric TDP-43 NTD can adopt a ubiquitin-like or DIX-domain-like fold in solution. Intriguingly, while several recent studies highlighted the importance of NTD for functional TDP-43 dimerization and nucleic acid interaction, others argued that the same domain promoted pathologic cytoplasmic aggregation and neurotoxicity. Moreover, a small fraction of TDP-43 was reported to exist as dimers in cells, which led to speculation that TDP-43 dimers may initiate or 'seed' the formation of high molecular weight pathologic TDP-43 aggregates.

We showed that physiological TDP-43 exists as nuclear oligomers that are distinct from cytoplasmic complexes formed upon cellular stress or pathologic aggregates. To elucidate the molecular basis of physiological TDP-43 oligomerization, we determined the crystal structure of TDP-43 NTD at 2.1 Å resolution, which revealed an unprecedented mode of head-to-tail interactions between monomers generating solenoid-like polymers. Consistent with the crystal structure, solution NMR spectroscopy confirmed the dynamic nature of inter-molecular and low micromolar affinity electrostatic interactions that stabilize these polymers. Destabilizing oligomerization by point mutations resulted in loss of TDP-43 regulation of alternative splicing of known neuronal RNA targets, indicating that these dynamic TDP-43 oligomers are the functional form of the protein in vivo. Tripartite GFP complementation experiments in cells illustrate that physiological TDP-43 oligomerization prevents LCD intermolecular interactions. Importantly, we show that NTD-driven TDP-43 oligomerization antagonizes pathologic aggregation. This dynamic head-to-tail polymerization of TDP-43, which is reminiscent of DIshevelled and aXin (DIX) domains involved in Wnt signaling, is unique among RNA-binding proteins (RBPs) and broadens our understanding of TDP-43 function.

Most excitingly, our findings indicate that stabilization of functional TDP-43 oligomers could have therapeutic potential by counteracting pathologic aggregation and restoring nuclear function. This work is currently in press in Nature Communications.

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C52 TDP-43 splicing repression: target identification and validation

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Keywords: TDP-43 splicing repression, AAV9 gene therapy, target validation

Identification of novel target for development of mechanism-based therapy for ALS and FTD remains a great unmet need. A highly conserved and essential RNA-binding protein, TDP-43, is cleared from the nucleus to form cytoplasmic inclusions, a pathology thought to play a central role in nearly all cases of ALS and a large proportion of FTD. While TDP-43 pathology may be an important therapeutic target, the lack of clarity regarding its pathogenic role hampers the ability to develop mechanism-based therapeutic strategy.

In contrast to regulating the usage of alternative exons, we discovered that by binding to simple microsatellite repeats, TDP-43 repressed the splicing of non-conserved cryptic exons, a function that is compromised in brains of ALS and FTD. Depletion of TDP-43 would result in the incorporation of cryptic exons that is predicted to negatively impact on both the transcriptome and the proteome of affected neurons. As several putative functions have been proposed for TDP-43 over the past decade, it will be important to target TDP-43 repression to establish that splicing repression is a major role served by TDP-43 in motor neurons. Our initial rescue of TDP-43 deficient cells from death using a chimeric protein comprised of the N-terminal RNA-recognition domain of TDP-43 fused with a well-studied but unrelated splicing repressor (called RAVER1) supported this notion. To establish this idea
in post-mitotic motor neurons, we employed both fly and mouse models lacking TDP-43 in motor neurons that exhibit an age-dependent motor neuron disease. Incorporation of cryptic exons as expected occurred in these flies or mice deficient in TDP-43.

Compared to flies lacking TDP-43, TDP-43 deficient flies expressing a TDP-RAVER1 construct in motor neurons showed repression of cryptic exons accompanied by marked increase in mobility and extension of lifespan. These outcomes strongly support the idea that the repression of splicing is a major role of TDP-43 in motor neurons. To validate TDP-43 repression as a therapeutic target, we employed an AAV9 viral vector to deliver this chimeric repressor in mice lacking TDP-43 in their motor neurons. We showed that such strategy could maintain long-term accumulation of TDP-RAVER1 in 55–70% of lower motor neurons without overt toxicity. Mice lacking TDP-43 in motor neurons infected with AAV9 expressing TDP-RAVER1 attenuated disease onset, slowed disease progression and led to an extension of lifespan. Importantly, TDP-RAVER1 prevented incorporation of cryptic exons. Together, these results establish that a major role of TDP-43 is to repress splicing in motor neurons, support the idea that compromised TDP-43 repression underlies neurodegeneration, and validate a potential AAV gene therapeutic strategy for the treatment of these devastating diseases of the elderly.

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C53 A bona fide TDP-43 knock-in mouse demonstrates perturbed TDP-43 regulation and helps yield candidate suppressors of cognitive dysfunction in ALS-FTD

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Keywords: TDP-43, FTD, Tau

Background: Amyotrophic lateral sclerosis-frontotemporal dementia (ALS-FTD) is characterised by TDP-43 pathology. Understanding how TDP-43 contributes to neurodegeneration will help direct therapeutic efforts. TDP-43 overexpression and knockdown approaches are problematic because TDP-43 demonstrates exquisite autoregulation (1) through alternative splicing of its 3’UTR. Experimentally increasing or decreasing TDP-43 levels both cause neurodegenerative phenotypes. However, to what extent human disease is caused by a gain and/or loss of TDP-43 function remains unclear.

Methods: To clarify the role of TDP-43 in the pathogenesis of ALS-FTD we used CRISPR/CAS9 to create a TDP-43 knock-in mouse harbouring only a human-equivalent point mutation in the endogenous mouse Tardbp gene (TDP-43Q331K). This model replicates the human mutant state as closely as possible, retaining the endogenous gene structure, and maintaining the essential and ubiquitous expression of TDP-43 both during development (2) and in adulthood. Mice were phenotyped using automated continuous behavioural monitoring (3), Rotarod, the 5-choice serial reaction time task, and using object recognition and marble burying assays. Histology and RNAseq of laser-captured spinal motor neurons and homogenized frontal cortex was additionally conducted.

Results: TDP-43Q331K mice display subtle motor impairment without neuromuscular denervation or significant motor neuron loss. However, mutant mice demonstrate executive dysfunction and hyperphagia, recapitulating features of FTD. No TDP-43 pathology is observed, but we identify loss of cortical parvalbumin interneurons in mutants. Surprisingly, immunoblot of frontal cortex demonstrates increased TDP-43 protein, while RNAseq identifies increased TDP-43 mRNA, indicating impaired autoregulation of TDP-43 expression, and leading to a gain of function as evidenced by altered splicing of Sort1. Furthermore, we identify increased inclusion of Mapt exons 2 and 3 in mutants. Finally, and most intriguingly, we identify phenotypic heterogeneity in mutants, which we exploit in a novel way by comparing the cortical transcriptomes of mice with more or less severe cognitive impairment. This reveals 471 changes linked with improved behaviour including down-regulation of two known modifiers of neurodegeneration, Atxn2 and Arid4a, and up-regulation of genes involved in translation and myelination.

Conclusions: With one base change in murine Tardbp, this study identifies TDP-43 misregulation as a pathogenic mechanism that may underpin ALS-FTD, adds to a growing body of evidence linking TDP-43 and Tau in ALS-FTD, and utilizes phenotypic heterogeneity to yield hundreds of candidate suppressors of neurodegeneration, thus accelerating progress towards identifying credible therapeutic targets for ALS-FTD.
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C54 Low expression of mutant ubiquilin-2 exacerbates ALS/FTD features in a TDP-43 mouse model
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Keywords: UBQLN2, TDP-43, mice model

Background: Mutations in the gene encoding Ubiquilin-2 (UBQLN2) are linked to amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). UBQLN2 plays a central role in ubiquitin proteasome system (UPS) and UBQLN2 mutants can form cytoplasmic aggregates in vitro and in vivo. We have recently demonstrated that UBQLN2 aggregates are dynamic structures that promote cytoplasmic accumulation of TAR DNA-binding protein (TDP-43), a major component of ALS inclusion bodies in neurons (1).

Objectives: There is an important lack of conclusive animal models in ALS research. The aim of the present study is to analyze mutations in both UBQLN2 and TDP-43 genes, which can increase cytosolic inclusion bodies, and lead to important motor and cognitive impairment in mice and mimic human ALS/FTD. This model will also be useful to study the in vivo role of UBQLN2 in TDP-43 mis-localization.

Methods: For this purpose, we have generated new transgenic hUBQLN2P497H/+ mice by micro-injection of flag-tagged human UBQLN2 gene under control of hNFH promoter in one-cell C57BL6 zygotes. hUBQLN2P497H/+ mice were then bred with our well characterized and acknowledged hTDP-43G348C/+ mouse model previously described in (2) to generate double transgenic hUBQLN2P497H/+;hTDP-43G348C/+ mice.

Results: The level of expression of our hUBQLN2 transgene was 20% out of the level of endogenous mUBQLN2, which supports the goal of a low expression model. Our double transgenic hUBQLN2P497H/+;hTDP-43G348C/+ mice developed important cognitive deficits at passive avoidance test at seven months of age. They exhibited an increase TDP-43 proteinopathy in spinal cord and brain at the age of 5 months. They also developed muscle atrophy and motor phenotype at a faster rate than the simple transgenic TDP-43G348C/+ mice.

Conclusions: These results suggested that UBQLN2 had a high tendency to induce mis-localization of TDP-43 and a slight disturbance in hUBQLN2 levels can trigger ALS pathogenesis. Double transgenic hUBQLN2P497H/+;hTDP-43G348C/+ mice developed typical features of ALS/FTD and could be exploited as a better animal model to test therapeutic avenue.

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C55 Unregulated expression of TDP-43 leads to divergent neurodegeneration in cortex and spinal cord in mice
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Keywords: TDP-43, transgenic mice, motor neuron

Background: Mutations in TDP-43 gene cause TDP-43 aggregation and familial ALS. Aggregation of wild-type TDP-43 is widely observed in sporadic ALS and FTD but its role in pathogenesis remains unclear. Both a gain and a loss of function may contribute to the pathogenesis. Some evidence suggests a modest elevation in wild-type TDP-43 level may be associated with sporadic ALS. Previous efforts in modeling an elevated level of TDP-43 in mice resulted in either early postnatal death or mild non-progressive phenotypes due to either too much or too little overexpression, respectively.

Methods: To determine the effects of a modest elevation of TDP-43 level, we have constructed a transgenic mouse model where the protein levels were increased by 10–20% in the hemizygotes and by 20–40% in the homozygotes in the CNS.
Results: The hemizygotes developed a progressive weakness ending in paralysis during the age of 500–700 days. This phenotype was not fully penetrant. The homozygotes developed the same but fully penetrant paralytic phenotype during the age of 300–500 days. We analyzed the homozygotes in detail. Cell quantification in motor cortex showed ~20% neuronal loss. The lost cells were mostly large pyramidal neurons. In the spinal cord, the most striking features were a severe demyelination associated with massive death of oligodendrocytes, a robust astrogliosis and microgliosis, and a dramatic elevation of neuroinflammation. Surprisingly, however, no motor neuron loss was detected although a modest denervation at the neuromuscular junction was present. Further analysis revealed that the TDP-43 transgene was primarily expressed in neurons, astrocytes and oligodendrocytes in the cortex, but only in astrocytes and oligodendrocytes in the spinal cord.

Discussion and conclusion: These results suggest that (1) a mild elevation of wild-type TDP-43 levels can cause age-dependent neurodegeneration in a cell-autonomous manner; (2) a severe demyelination and neuroinflammation can damage axons but is insufficient to kill MNs; and (3) the paralysis in these mice is likely caused by a combination of upper motor neuron loss and severe demyelination in the spinal cord. This new TDP-43 mouse model may be useful to study the effects of mild elevation of TDP-43 levels in the CNS cells and to test therapies targeting TDP-43 toxicity.

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C56 Presymptomatic lifestyle classified according to C9orf72 genotype

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Keywords: C9orf72 repeat expansion, presymptomatic lifestyle, gene-environment interaction

Background: Amyotrophic lateral sclerosis (ALS) is a complex disease caused by genetic and environmental factors, in a multistep fashion (1,2). Previous studies assessed the relationship between ALS and environmental risk factors or genetic factors, but gene-environment interactions have not yet been studied.

Objectives: To assess cross-sectional and longitudinal changes of presymptomatic lifestyle, stratified for subjects with and without a C9orf72 mutation.

Methods: 142 ALS patients with a C9orf72 mutation (carriers) and 1328 patients without a C9orf72 mutation (non-carriers) from a national population-based study participated (3). A total of 1328 population-based controls were used as reference and matched for age and gender. We cross-sectionally and longitudinally assessed the relationship between the C9orf72 mutation and presymptomatic physical activity in metabolic equivalents of task (MET) (4), smoking pack-years, cumulative alcohol consumption, caloric intake and body mass index (BMI) up to 50 years before onset and adjusted for relevant covariates.

Results: Compared to controls, pack-years and caloric intake were significantly higher, and alcohol intake lower for both carriers (4.03 pack-years, p = 0.003; 620 kJ, p = 0.025; –235 units, p = 0.010) and non-carriers (2.46 pack-years, p<0.001; 500 kJ, p<0.001; –73 units, p<0.001). The longitudinal rate of increase of the cumulative physical activity was higher in non-carriers than in controls (difference at onset (DaO): 322 METs, p<0.001), which was significant from 34.9 years before onset, and lower in carriers than in controls (DaO: –376 METs, p=0.02), which was significant from 11.5 years before onset. Relative to controls, BMI of carriers was decreasing over time, and this was significant from 32 years before symptom onset (DaO: –1.4, p<0.001). BMI of non-carriers was significantly higher 30–50 years before onset (mean 0.40, p<0.05), and turned significantly lower in the last 8 years before symptom onset (mean –0.38, p<0.05).

Discussion and conclusions: Presymptomatic lifestyle factors of ALS patients differ from controls, and C9orf72 mutation carriers can show opposite lifestyle compared to non-carriers. Differences between carriers, non-carriers and healthy subjects can be detected up to 50 years before first symptoms of weakness.

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C57 Genetic mutations shorten the multistep process in ALS

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Keywords: multistep process, genetic mutations

Background and aim: In a previous study, we utilized an approach derived from cancer research to assess whether amyotrophic lateral sclerosis (ALS) incidence is consistent with the causal disease mechanism being a multistep process (1). We found evidence to support a multistep hypothesis of ALS, and that the process leading to ALS needs on average six molecular steps. Here we test the hypothesis that carrying a large effect mutation might account for one or more of the steps through the effect of the mutation, and thus leave fewer remaining steps before ALS begins.

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Methods: We collected incidence data of an ALS population register in Italy (2007–2015) for which genetic analysis for C9orf72, SOD1, TARDBP and FUS genes were generated. As confirmation, we used data from ALS cases diagnosed in the Republic of Ireland in the period 2006–2014. The log of age-specific incidence against the log of age with least squares regression for the subpopulation carrying disease-associated variation in each separate gene was regressed, using the regression coefficient and slope parameters to assess the evidence for a multistep model.

Results: We identified 1075 cases of ALS with genetic test data (80% of all incident patients). C9orf72 mutations were detected in 73 cases (6.8%), SOD1 in 20 (1.9%), TARDBP in 15 (1.4%) and FUS in 3 (0.3%). In the 1075 patients with genetic test data, there was a linear relationship between log(incidence) and log(age) \( r^2 = 0.98 \) with a slope estimate of 4.99, consistent with a 6-step process. The analysis for C9orf72 mutated patients confirmed a linear relationship \( r^2 = 0.94 \) with a slope estimate of 2.22 suggesting a 3-step process. This estimate was confirmed by the data of the Irish ALS register. A linear relationship was found for SOD1 mutated patients \( r^2 = 0.53; n-1 = 0.76 \) consistent with a 2-step process, and for TARDBP \( r^2 = 0.94; n-1 = 3.24 \) consistent with a 4-step process.

Conclusions: The identification of a reduced number of steps in ALS patients with genetic mutations compared to those without mutations strongly supports the idea of ALS as a multistep process and is an important advance for dissecting the pathogenic process in ALS.

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C58 Amyotrophic lateral sclerosis and food intake in Italy

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Keywords: diet, case-control, Italy

Background: Many dietary factors have been investigated to verify their role in the onset or the progression of the disease. Vitamins, proteins, amino acids and other nutrients have been studied without showing clear beneficial or adverse effects. Inadequate study designs, differing target populations, and lack of statistical power are possible explanations. In addition, there are no studies focusing on specific nutrients in ALS.

Objectives: The aim of the study was to verify if specific (combinations of) foods and nutrients could be risk factors or protective factors in the development of ALS.

Methods: Patients with newly diagnosed ALS residing in 3 Italian administrative regions (Lombardy, Piedmont and Valle D’Aosta, Puglia; total population 18,629,052) were included in a European prospective observational study from February 2011 through January 2015 as part of the Euro_MOTOR collaboration. Patients were included if they were aged 18 or older and had definite, probable or possible ALS according to the El Escorial (EE) diagnostic classification. For each patient, a healthy control, matched for age (±5 years), sex and administrative region of residence, was selected by a general practitioner (1:1 ratio). After signed informed consent, cases and controls were interviewed by a trained investigator who filled a validated and reproducible food-frequency questionnaire (FFQ). Daily intake of macronutrients (carbohydrates, ie sugars and starch, protein, fat, cholesterol, and fibres), micronutrients (vitamins and minerals), fatty acids, and total energy were estimated using an Italian food composition database. 212 cases and 212 matched controls were included in the study (females, 188; males 236; mean age at diagnosis 62.6 years; SD 10.4 years). ALSFRS-R score at admission, 37.3 (SD 8.7).

Results: A risk reduction was found for coffee and tea (OR = 0.29, 95% CI 0.14–0.60 for the highest versus the lowest quartile of consumption), whole bread (OR = 0.55, 95% CI 0.31–0.99), raw vegetables (OR = 0.25, 95% CI 0.13–0.52) and citrus fruits (OR = 0.49, 95% CI 0.25–0.97). After separating coffee and tea into subgroups, a significant risk reduction was observed for decaffeinated coffee (OR = 0.18, 95% CI 0.06–0.57) and for tea (OR = 0.36, 95% CI 0.19–0.67), but not for coffee (excluding decaffeinated coffee). A risk increase was observed for red meat (OR = 2.96, 95% CI 1.46–5.99) and pork and processed meat (OR = 3.87, 95% CI 1.86–8.07). A significant inverse association was observed for vegetable fat (OR = 0.25, 95% CI 0.11–0.57 for the highest versus the lowest quartile of intake), total folate (OR = 0.41, 95% CI 0.18–0.91), and vitamin E (OR = 0.40, 95% CI 0.18–0.92). An increased risk was found for total protein (OR = 2.96, 95% CI 1.08–8.10), animal protein (OR = 2.91, 95% CI 1.33–6.38), sodium (OR = 3.96, 95% CI 1.45–10.84) and zinc (OR = 2.78, 95% CI 1.01–7.83). A risk increase was also found for the highest versus the lowest quartile of glutamic acid intake (OR = 3.63, 95% CI 1.08–12.2).

Discussion: Our findings support the hypothesis that some foods/nutrients may be risk factors and others protective factors for ALS. However, a definite answer to this controversial issue could be provided only by a larger and specific evaluation of few nutrients matched with specific genetic tests.

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**C59 The effects of duration and intensity of cigarette smoking on the risk of amyotrophic lateral sclerosis**

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Keywords: risk factor, smoking, epidemiology

**Background:** The aetiology of amyotrophic lateral sclerosis (ALS) is largely unknown. Pre-existing genetic load is thought to be acted upon by time and environmental exposures until a tipping point is reached and neurodegeneration begins. A wide range of potential exogenous risk factors for ALS have been studied, but as yet only cigarette smoke has been considered a probable risk factor. Although results have not been conclusive, there seems to be a clear association between the number of years exposed to cigarette smoke and the risk of ALS. We further investigated the association between smoking and the risk of ALS in a population-based study by exploring the effect of intensity, duration and time since cessation of smoking.

**Methods:** ALS cases and matched controls were recruited from the Netherlands (2006–2015). Demographics and detailed lifetime smoking histories and information on other lifestyle factors, up to two years before survey date, were collected via paper questionnaires. Odds ratios (OR) and 95% confidence intervals (CI) were derived through logistic regression models for smoking status, intensity (cigarettes per day), duration (in years), cigarette pack-years and time since cessation (in years). Models were adjusted for age, sex, alcohol drinking status and education. The reference group consisted of never smokers. Categories were based on the quartiles of exposure distribution among controls.

**Results:** Overall, 1548 cases and 3952 controls with complete smoking histories were included. The OR for current smokers was 1.59 (95% CI 1.27–2.00) compared with never smokers. Smoking pack-years was positively associated with increased risk of ALS ($p=0.002$). This association appeared to be predominantly driven by smoking duration: the model for duration showed a clear positive trend with ($p<0.0001$) while average smoking intensity did not ($p=0.162$). Time since cessation of smoking showed a clear inverse relation with the risk of ALS ($p<0.0001$).

**Conclusion:** Our findings within this large case-control study provide further support for the causal association between smoking and ALS. We showed a clear increasing risk of ALS with the number of years smoked and a decreased risk with time since cessation. Understanding the dynamics of the effect of smoking on the risk of ALS may inform hypotheses on the disease mechanism and will improve understanding of the aetiology of ALS.

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**C60 Spatial analysis of amyotrophic lateral sclerosis (ALS) cases in the United States and their proximity to multidisciplinary ALS clinics, 2013**

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Keywords: spatial analysis, multidisciplinary clinics, case proximity, access to care

**Background:** Multidisciplinary ALS clinics (MDCs), often considered the gold standard of ALS care, have been established to provide specialty symptomatic and palliative care to people living with this terminal disease with limited treatment options.

**Objective:** The aim of this analysis is to provide a spatial representation of ALS prevalence cases to the nearest MDCs across the U.S. to help determine proximity to care.

**Methods:** The 2013 prevalence data from the National ALS Registry (Registry) were used for this spatial analysis. Cases were geocoded and plotted on a map by city of enrollment in the Registry by using geographic information system (GIS) software, along with the locations of all MDCs in operation during 2013. For regional analysis, the U.S. was grouped into four regions as specified by the U.S. Census Bureau: Northeast, South, Midwest, and West. Case-to-MDC proximity was calculated and analyzed by sex, race, and age group.

**Results:** Of the 15,908 total ALS cases identified in 2013 through the Registry, 98.3% (15,633) had geocodable cities for mapping and the cases were distributed in all 50 states. Of these 15,633 cases, 62.6% were male, 77.9% were white, and 76.2% were in the 50–79 years age range. The combined individual states with the greatest number of cases were California, Florida and Texas (3436, 22.0%). During 2013, there were 71 MDCs in operation in 30 different states. For overall case-to-MDC proximity, 37.9% (5918) of the geocodable ALS cases lived within 0–25 miles of an MDC, followed by 24.6% (3841) who...
lived >100 miles. There was a statistically significant difference between distance grouping to MDC among Whites vs. Blacks and Whites vs. Others (p<0.0001, respectively), in addition to Blacks vs. Others (p<0.05). Additionally, there were statistically significant differences for distance groupings among those 18–49 vs. ≥80 years (p<0.05), and 50–79 vs. ≥80 (p<0.05), but no significant difference for those 18–49 vs. 50–79 years (p = 0.75). There was also no statistically significant difference among case-to-MDC proximity by sex.

Discussion and conclusions: This spatial analysis is the first to examine the proximity of population-based ALS prevalence cases for the entire U.S. to MDCs. Of the approximately 16,000 people with ALS in the U.S., almost half live >50 miles from an MDC. Having better access to care, whether at MDCs or through other modalities, is likely key to increasing survivability and obtaining appropriate end-of-life treatment and support for people with ALS.

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Session 7C Emerging Markers

C61 Imaging denervation in amyotrophic lateral sclerosis for clinical trials: a longitudinal cohort study

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Keywords: MRI, MUNIX, muscle

Objective: To assess the utility of whole-body MRI to quantify muscle denervation longitudinally in patients with amyotrophic lateral sclerosis (ALS), as a tool for future clinical trials, and to probe pathophysiological mechanisms in vivo.

Methods: A prospective longitudinal observational cohort study was performed. 29 ALS patients and 22 age and sex-matched healthy volunteers were assessed with clinical measures (revised amyotrophic functional rating scale (ALSFRS-R; individual muscle MRC scores), electrophysiological motor unit number index (MUNIX) and T2-weighted whole-body muscle MRI, at first clinic presentation and four months later. Between-group differences and associations were assessed using multivariable regression models, adjusted for age and gender. Within-subject longitudinal changes were assessed using paired t-tests. Patterns of disease spread were modeled using mixed effects multivariable regression, assessing associations between muscle relative T2 signal and anatomical adjacency to site of clinical onset.

Results: ALS patients had 30% higher relative T2 muscle signal than healthy volunteers at baseline (all-regions mean, 95%CI 15%–45%, p<0.001). In patients, higher mean all-regions T2 signal was associated with greater disability on ALSFRS-R (coefficient –0.009, 95% CI –0.017 to 0.001, p = 0.023). Higher individual muscle T2 signal was associated with clinical weakness and lower MUNIX in biceps (p = 0.024 and p = 0.015, respectively) and tibialis anterior (p<0.001 and p = 0.003, respectively). Relative T2 signal in bilateral tibialis anterior increased over four months in ALS patients (right: 10.2%, 95% CI 2.0%–18.4%, p = 0.017; left: 14.1%, 95% CI 3.4%–24.9%, p = 0.013). There was no support for anatomically contiguous disease spread from site of onset on MRI in this model.

Conclusions: Whole-body muscle MRI offers a new approach to the objective assessment of denervation over short timescales in ALS, and enables investigation of patterns of disease spread in vivo. Muscles inaccessible to conventional clinical and electrophysiological assessment may be investigated.

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C62 Cortical excitability index: a novel diagnostic biomarker in ALS

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Keywords: diagnosis, transcranial magnetic stimulation, upper motor neuron biomarker

Objective: To assess the sensitivity and specificity of a novel upper motor neuron biomarker, the cortical excitability, in sporadic amyotrophic lateral sclerosis (ALS).

Methods: A large prospective study recruiting 407 patients (245 males, 162 females) was performed in accordance with standards of reporting of diagnostic accuracy criteria to assess the diagnostic utility of a novel cortical excitability score in ALS. The study utilized a study sample of 305 ALS patients and 102 non-ALS neuromuscular mimic disorder.

Results: The median cortical excitability score performed significantly better in ALS patients compared to neuromuscular controls (p<0.0001). There was a robust area under curve of 0.93, indicating an ‘excellent’ diagnostic utility. The number needed to test in order to diagnose one extra case of ALS was 1.3 (1.1–1.6). Importantly, an extra 39% of patients could be diagnosed with ALS when applying the cortical excitability score to the Awaji criteria, and an extra 16% of patients when applied to the Ludolph’s criteria. The diagnostic utility of the cortical excitability score was independent of therapy
status, site of disease onset or presence of upper motor neuron signs.

Conclusion: The present study established a diagnostic utility of the cortical excitability score in sporadic ALS, enhancing the diagnostic utility of the Awaji and Ludolph's criteria. The cortical excitability score may be a novel biomarker allowing incorporation into the diagnostic algorithm for ALS in clinical practice and therapeutic trials.

Classification of Evidence: The study provides Class I evidence for diagnostic utility of the cortical excitability score in sporadic ALS.

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C63 Cortical dysfunction appears to be a regional feature in ALS

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Keywords: onset, progression, cortical hyperexcitability

Introduction: Cortical hyperexcitability is an early and reliable biomarker of corticomotorneuronal dysfunction in ALS, preceding the onset of lower motor neuron degeneration. Importantly, cortical hyperexcitability appears to be more prominent when assessed in the hemisphere contralateral to symptom onset, implying a role in disease spread, although recordings have only been conducted over the thenar eminence, thereby precluding definitive conclusions.

Aim: The aim of the present study was to assess regional variations in cortical hyperexcitability and relate these to patterns of disease spread in a large cohort of ALS patients.

Methods: Threshold tracking transcranial magnetic stimulation (TMS) studies were undertaken in 48 ALS patients (26 males, 22 females, mean age 63 years) with responses recorded over the abductor pollicis brevis (APB), tibialis anterior (TA) and trapezius muscles, representing three separate regions. Results were compared to 20 age matched controls. All patients underwent detailed clinical and neurophysiological assessment in order to establish a diagnosis of ALS as per the Awaji diagnostic criteria.

Results: Cortical hyperexcitability was evident in all regions recorded: upper limb (APB), lower limb (TA) and bulbar (trapezius). Importantly, cortical hyperexcitability was most prominent in the upper limbs as indicated by reduction in short interval intracortical inhibition (SICI) (p<0.01) and cortical silent period (CSP) duration (p<0.05), as well as increases in MEP amplitude (p<0.01). Although cortical hyperexcitability was evident in the lower limbs and bulbar regions, it was not as prominent as in the upper limbs. Separately, motor cortex inexcitability appeared to also be more common in the lower limb (56%) and bulbar (40%) regions.

Conclusion: Cortical hyperexcitability appears to be a regional phenomenon in ALS, being most prominent in cortical areas representing the hand region. Given that the hand region is the most frequently reported site of disease onset in ALS, the present findings may suggest the importance of cortical hyperexcitability in mediating disease onset in ALS. Strategies aimed at modulating cortical hyperexcitability in early stages of ALS may prove therapeutically useful.

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C64 Metabolic changes in asymptomatic C9orf72 carriers compared with non-carriers in the same family assessed by brain 7T MRSI

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Keywords: magnetic resonance spectroscopic imaging, C9orf72 repeat expansion, presymptomatic

Background: The earliest disease effects might be detected in brain metabolism of asymptomatic subjects at high risk of developing ALS (1). Asymptomatic subjects with a C9orf72 repeat expansion (carriers) were previously shown to have morphometric brain alterations compared to non-carriers but underlying metabolic changes are currently unknown (2).

Objectives: To investigate changes of brain metabolism in pre-symptomatic carriers compared with non-carriers.

Methods: In vivo imaging of brain metabolism was performed using proton magnetic resonance spectroscopic imaging (MRSI) to compare brain metabolism of 11 asymptomatic C9orf72 carriers compared with 18 non-carriers from the same large family with a history of C9orf72 related ALS. High resolution MRSI data of the brain were obtained using a 7 Tesla MR scanner. MRSI data were acquired using a 44 x 44 matrix (voxels: 5 x 5 x 10 mm³) intersecting the brain regions that were previously shown to have morphometric brain alterations (2). Post-processing was performed in Matlab (Mathworks Inc.), and LCModel was used to fit the spectra and calculate the metabolite ratios (3). Only voxels that passed a semi-automated quality control were included in the statistical analysis. Spectra of 6 brain regions were
compared between carriers and non-carriers, and random-effects meta-analyses comprising all studied brain regions were performed to assess global involvement of brain metabolism.

**Results:** Carriers had a lower N-acetylaspartate + N-acetylaspartylglutamate (tNAA) to phosphocreatine + creatine (tCr) ratio (tNAA/tCr) in the left putamen (–0.27, 95% confidence interval (95% CI) –0.51 to –0.03, \(p=0.03\)) than non-carriers. The glutamate to tCr ratio (Glu/tCr) was also lower in the left putamen of carriers compared to non-carriers (–0.28, 95% CI –0.50 to –0.07, \(p=0.009\)). To assess differences of glutamate concentration corrected for neuronal density, we compared the Glu/tNAA ratio between carriers and non-carriers. This ratio was lower in carriers than in non-carriers in the left putamen (–0.08, 95% CI –0.14 to –0.02, \(p=0.027\)). Using meta-analyses, we combined outcomes of the six regions and showed lower tNAA/Glu (\(p=0.012\)), Glu/tCr (\(p=0.031\)) and tNAA/tCr ratios (\(p=0.081\)) in C9orf72 carriers.

**Discussion and conclusions:** We showed that the C9orf72 repeat expansion affects brain metabolism of asymptomatic subjects at high risk for developing ALS. The pattern of involved brain metabolites suggests that carriers have a lower concentration of neurons and a lower glutamate concentration per neuron compared to non-carriers. These metabolic alterations may be important biomarkers to monitor treatment effects in future trials in pre-symptomatic carriers and might hold clues for the pathologic processes underlying (C9orf72-related) ALS.

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**C65 The metabolic signature of ApoE genotype in ALS: a \(^{18}\)F-FDG-PET study**

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**Keywords:** ApoE, cognition, \(^{18}\)FDG-PET imaging

**Background and aim:** In Alzheimer’s dementia (AD) the \(e2\) allele of the ApoE gene lowers the risk and delays the onset of the disease. Conversely the \(e4\) isoform represents a risk factor, increasing the incidence of dementia by ~3-fold in heterozygous carriers and 12-fold in homozygous carriers. We recently investigated the effect of ApoE genotype on the risk of cognitive impairment in a population-based series of ALS patients, collected through the Piemonte and Valle d’Aosta Register for ALS. We found that \(e2\) allele provides an increased risk of FTD (1). The aim of the present study was to evaluate the metabolic correlates of the ApoE genotype in ALS patients.

**Methods:** The ApoE genotype (from \(e2/e2\) to \(e4/e4\)) was regressed in 159 ALS patients against whole brain metabolism as assessed by \(^{18}\)F-FDG-PET. SPM8 Multiple Regression routine was implemented with age, sex, education and type of onset as covariates. Statistical significance threshold was set at \(p<0.005\) uncorrected.

**Results:** Higher metabolism positively correlated with genotype lacking \(e2\) alleles in correspondence with bilateral frontal, prefrontal, orbitofrontal and anterior cingulate cortices and in the right thalamus. No significant negative correlation was detected.

**Conclusion:** The presence of \(e2\) allele in ALS patients is associated with a lower metabolism in brain areas related to cognitive impairment. These data support our previous finding of a role of the \(e2\) isoform of ApoE in increasing the risk of frontal cognitive deficits in patients suffering from motor neuron disease.

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C66 Characterisation of a novel ALS-associated candidate gene identified from whole exome sequencing

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Keywords: candidate gene, whole exome sequencing, protein aggregation

Background: The development of whole exome sequencing (WES) has led to acceleration in deciphering the genetic architecture of Mendelian disorders such as amyotrophic lateral sclerosis (ALS). In 2016–2017, 3 novel genes including NEK1, CCNF and ANXA11 have been discovered to be associated with ALS through WES, reinforcing the power of this technology. To identify novel candidate genes, we have performed WES in a total cohort size of 1008 ALS patients, including 750 index cases with 68 affected relatives for which the pathogenic mutation has yet to be identified.

Objectives: The objective was to identify novel ALS candidate genes and characterise the mutations.

Method: Due to lack of large kindreds from which to perform linkage analysis, variants from next generation reads were filtered to those that are novel and absent from ExAC, EVS, UK10K, 670 local control exomes (n = 70,000). To confirm the novelty of these variants, subsequent direct sequencing was performed on over 2000 ALS cases and 1000 controls of U.K., U.S. and Italian origin. Modelling of these mutations was performed by overexpressing the human cDNA containing either the wild-type or point mutations in HEK293T cells and primary rat cortical neurons.

Results: As a proof of principle, our analysis has identified seven known disease-causing mutations in common ALS genes including SOD1, FUS and TARDBP. Additionally, we have identified mutations shared by several index cases in the ARPP21 gene, a novel candidate gene that is highly expressed in the central nervous system. However, little is known about its functions and localisation. An extensive in-house screening has further identified several other mutations in the disordered region of low sequence complexity similar to other ALS genes including TARDBP and FUS. In vitro overexpression studies demonstrated that ALS-associated mutants recapitulate the pathological hallmark of ALS including detergent insoluble aggregates, colocalisation with cytoplasmic TDP43 granules as well as impaired protein degradation.

Discussion and conclusions: By combining the genetics and follow-up functional assessment, we believe that this is the most promising approach to identify causative genes in cases with unknown genetic cause and uncover underlying mechanism.

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C67 Targeted genetic screen of RNA-binding proteins in amyotrophic lateral sclerosis reveals novel genetic variants with synergistic effect on clinical phenotype

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Keywords: RNA binding proteins, C9orf72, genomics

Background: Amyotrophic lateral sclerosis (ALS) is underpinned by a polygenic rare variant architecture. Identified genetic variants of ALS include RNA-binding proteins containing prion-like domains (PrLDs). We hypothesized that screening related proteins will yield novel genetic variants of ALS. In the most common genetic variant of ALS patients carry a G4C2-repeat expansion within C9orf72. We have shown that G4C2-repeat RNA sequesters RNA-binding proteins. A logical consequence of this is that loss-of-function mutations in G4C2-binding partners might contribute to ALS pathogenesis independently of and/or synergistically with C9orf72 expansions.

Objectives: To perform targeted sequencing of genomic DNA encoding RNA-binding proteins in ALS patients in order to identify novel genetic variants and explore genotype-phenotype relationships.

Methods: Genomic DNA was extracted from 109 ALS patients including 32 familial ALS patients, 61 young-onset sporadic ALS patients and 15 C9orf72-ALS patients. Genomic DNA was enriched for selected RNA-binding proteins and known genetic variants of ALS using a custom design Agilent SureSelect in solution kit.
Sequencing was performed using an Illumina HiScan platform according to manufacturer’s instructions. Rare deleterious mutations were defined by frequency within the Exome Aggregation Consortium data set of <1/10,000 controls, and a Phred-scaled Combined Annotation Dependent Depletion score >10 (change is within 1% of most deleterious reference variants). We validated changes with low read depth by Sanger sequencing.

**Results:** We identified 59 patients with a rare deleterious mutation of which 14 carried more than 1 mutation. The frequency of identified mutations in familial ALS patients was higher than in sporadic ALS patients (72% compared to 59%) as expected.

We identified new mutations in 11 known ALS genetic variants which served as a validation of our strategy. We identified 18 patients with at least one mutation in a RNA-binding protein containing a PrLD. The number of mutations per patient correlated with rate of disease progression (Pearson correlation coefficient $-0.62$, $p = 0.0097$). There was no additional synergy in combination with C9orf72 expansion. We identified 21 patients with a single mutation in a G4C2-repeat binding protein. Five patients also carried a G4C2-repeat expansion in C9orf72. Patients with a G4C2-binding protein mutation in combination with a C9orf72 expansion had a significantly faster disease course (Pearson correlation coefficient $-0.57$, $p = 0.0084$).

**Discussion:** Our data are consistent with a polygenic model of ALS in which multiple rare variants act collectively to cause disease. We provide evidence for a number of entirely novel genetic variants of ALS caused by mutations in RNA-binding proteins. Moreover, we show that these mutations act synergistically with each other and with C9orf72 expansions to modify the clinical phenotype of ALS. This work has significant implications for ALS therapy development.

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**C68 Integrating copy number analysis with structural variation detection in whole genome sequenced ALS UK cohort**

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**Keywords:** copy number variation, structural variation, whole genome sequencing

**Background:** Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease predominantly of motor neurons, characterized by progressive weakness of voluntary muscles and death from respiratory failure due to diaphragmatic paralysis, typically within three years of onset. Despite the very poor prognosis, there is considerable variation in the survival rate, and up to 10% of people with ALS live more than eight years from onset of first symptoms. There is a strong genetic contribution to ALS risk. In 5% of cases a family history of ALS or frontotemporal dementia is obtained. The Mendelian genes responsible for ALS in such families have now been identified in more than 70% of cases. Even in apparently sporadic cases, twin and population studies have shown the heritability is still about 60%.

Although risk genes reveal information about the possible mechanism of causation in ALS, it is also important to identify gene variants that modify survival. Survival genes could potentially be targeted directly, or their product augmented to improve ALS survival.

A number of common gene variants associated with ALS survival have been identified through genome-wide association studies and other genome-wide approaches such as structural variants.

**Methods:** Copy Number Segmentation was performed by Regression Tree in Next Generation Sequencing. This was applied to whole-genome sequencing data from 1400 ALS patients. Mapping of the copy-number structural variation was quantified and mapped to chromosomal position using CREST.

**Results:** Up to 2500 copy number variations were identified; of these 84.6% of variations were deletions, 9.70% were insertions and the least observed variations were inter-chromosomal translocations.

**Conclusions:** Deletion was the most common variation observed in ALS cases. Chromosomes 13 and 17 show the highest structural variations in ALS. About 90% of inter-chromosomal translocation events were involved within chromosome 13 and 15.

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**C69 Genome wide association study of genetic modifiers in ALS carriers of repeat expansions in C9orf72 gene**

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Keywords: genome wide association study, Cox proportional hazards regression analysis, C9orf72 gene

Background: Nearly 10% of patients with sporadic ALS carry a hexanucleotide expansion in the C9orf72 gene. Although ALS C9orf72 expansion carriers are reported to have a lower age of symptom onset and reduced survival compared to non-expansion ALS patients (1), these sub-phenotypes do not correlate with C9orf72 repeat expansion size (2) and factors that determine the large clinical variability in these phenotypes still remain unknown.

Objectives: Our aim was to identify genetic factors and their pathways influencing age at symptom onset and survival in ALS C9orf72 expansion carriers. Secondly, we analysed the genetic relationship between survival and age of onset in ALS C9orf72 expansion carriers and in non-carriers using linkage disequilibrium score regression.

Methods: Participants and GWAS data: ALS C9orf72 expansion carriers were drawn from the SLAGEN Consortium (87 newly genotyped Italian patients) and from samples collected by the Project MinE and STRENGTH Consortia (285 C9orf72 expansion carriers). Case-control genotypes were cleaned and imputed separately in each platform and then combined in a unique pipeline. Data were extracted and tested for duplicates and population stratification.

Statistical analyses: For survival, clinical information was available for 364 C9orf72 expansion carriers (85.5% deceased). We employed multivariate Cox proportional hazards regression analysis and, for variants achieving genome-wide significance, a dominant genetic model, Kaplan-Meier and log rank analyses were used. Age at onset was analysed by linear regression analysis adjusted for the first principle component in 371 C9orf72 expansion carriers. Gene-set analysis was used in both survival and age at onset analyses. Genetic correlations between ALS C9orf72 expansion carriers and in non-carriers for survival and age of onset were estimated by linkage disequilibrium score regression using the restricted maximum likelihood method (CGTA).

Results: 2 loci were genome wide significantly associated with survival in ALS C9orf72 expansion carriers: at 5q35, \( p = 3.6 \times 10^{-8} \), and at 10p13, \( p = 4.1 \times 10^{-8} \). Associated variants were rare. For age at onset regression analysis, 3 loci were genome wide significantly associated. At 6p21 3 intronic SNPs \( p = 4.68 \times 10^{-10} \), 1.53 x 10^{-9}; 9.79 \times 10^{-08} \) with MAF = 0.036 were associated with an earlier disease onset of \( \sim 10 \) years. A further 2 loci, at 10q22 and 15p13, were also genome wide significantly associated.

Discussion and conclusions: We have identified novel rare loci significantly associated with lower age at onset and shorter survival in a cohort of ALS C9orf72 expansion carriers, confirming a role for rare variation in the complex genetic architecture of ALS and focusing on the need for larger samples with full genome coverage. We identified 2 pathways that provide additional insight into biological mechanisms that regulate age at onset in ALS C9orf72 expansion carriers suggesting novel targets for drug treatment.

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C70 DNA methylation age-acceleration is associated with disease duration and age at onset in C9orf72 patients

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Keywords: C9orf72, DNA methylation age, epigenetics

Background: The repeat expansion in C9orf72 is the most common cause of amyotrophic lateral sclerosis and frontotemporal dementia. C9orf72 patients present with a wide range in disease duration and age of onset. The strongest risk factor for both syndromes is aging, which was linked to DNA methylation (DNAm) age based on the cumulative assessment of the methylation levels of 353 CpGs included on the genome-wide 450k BeadChip. DNAm age may reflect biological age better than chronological age.

Results: We conducted a genome-wide blood DNA methylation study of 46 unrelated C9orf72 patients. After correction for multiple testing, none of the CpGs demonstrated association between its methylation level and disease duration or age of onset. However, we detected a significant reverse correlation of DNAm age-acceleration with disease duration and age of onset, suggesting that for every 5-year increase in DNAm age-
acceleration there is a 3.2-year earlier age of onset and a 1.5-year shorter disease duration. The significant correlations remain after adjusting for gender, TMEM106B genotypes, disease phenotype and C9orf72 5'CpG island methylation status. The significant correlations remain after adjusting for gender, TMEM106B genotypes, disease phenotype and C9orf72 5'CpG island methylation status. A similar trend was observed for the blood DNA of affected members of an extended C9orf72 family; and tissues from the central nervous system of C9orf72 autopsy cases. For instance, regression analysis suggested that a 5-year increase in DNAm age-acceleration is linked to an earlier age of onset by 4.7 years or 5.5 years for frontal cortex or spinal cord, respectively. Blood DNAm age may be a useful biomarker for biological age, because blood DNAm age-acceleration was similar to all investigated brain tissues, except for cerebellum that ages more slowly.

Discussion: In conclusion, DNA methylation analysis of C9orf72 patients revealed that increased DNAm age-acceleration is associated with a more severe disease phenotype with a shorter disease duration and earlier age of onset.

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C71 Discovery of previously unknown relationships between ALS patients increases power to identify causal disease genes

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Keywords: relatedness, genome, exome

Background: Approximately 10% of ALS cases are hereditary (familial ALS, FALS), with the remaining cases occurring as apparently sporadic disease (SALS). To date, the only proven cause of ALS are gene mutations that lead to motor neuron death, and the majority of these causal mutations were discovered in familial ALS. However, remaining families often exhibit reduced disease penetrance, sometimes blurring the distinction with apparently sporadic ALS. Similarly, when an ALS-linked mutation is identified in an apparently sporadic case, genealogical analysis will occasionally detect a family history of ALS. With large ALS patient sequencing datasets available, we have an opportunity to detect these previously unknown relationships, increasing the power of genetic studies to identify new ALS genes.

Objectives: We sought to identify previously unknown relationships within a large cohort of Australian familial and sporadic ALS cases, thereby increasing the power of genetic analysis to discover disease variants.

Methods: We analysed a cohort of 137 familial ALS cases from 94 families with known relationship structure, and 35 apparently sporadic ALS cases. Familial ALS cases included first-degree relatives (n = 32), second-degree relatives (n = 18), third-degree relatives (n = 11), fourth degree relatives (n = 2) and duplicates (n = 2). FALS cases underwent whole exome sequencing. Sporadic ALS cases underwent PCR-free whole genome sequencing. Relatedness analysis used the open-source software KING (1).

Results: KING relatedness analysis using all variants (n = 7,290,477) from the 137 FALS exome data and extracted exomes from the 35 SALS genomes could only detect 1/67 known relationships in our FALS cohort. We then determined a ‘common exome’ from the different library capture methods, comprising 38,212 variants genotyped in 100% of samples, and subsequently performed relatedness testing on this subset of variants for the full cohort of 172 samples. KING analysis of this subset of variants in the FALS cohort identified 65/65 known relationships. Extending the KING relatedness analysis to include the 35 SALS samples has identified 8 potential third-degree relatives, 5 between SALS and FALS samples and 3 within the SALS cohort. We have however ruled out 3 SALS-FALS relationships as false-positive due to mutation status mismatch. We are also developing our own tool for discovery of relationships within our ALS cohort, aiming to improve on the specificity of KING.

Discussion and conclusions: These potentially related individuals will proceed through an established gene discovery pipeline as new ALS relatives, to determine if they harbour novel shared candidate mutations. This strategy will be extended to >700 Australian SALS genomes whose WGS is nearing completion. Identification of new relationships between ALS patients can lead to disease gene discovery. Each new ALS gene can aid diagnosis and offer the chance to investigate the molecular mechanisms leading to neurodegeneration.

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C72 NEALS Bulbar Subcommittee: protocol design for speech and swallowing

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Keywords: bulbar, speech language pathologist, dysphagia

The prevalence of bulbar dysfunction in ALS significantly contributes to malnutrition, aspiration, pneumonia, reduced quality of life, and death. Validated clinical bulbar scales to assess disease progression are currently lacking. A recent bulbar practice survey of sites in the North East ALS (NEALS) consortium demonstrated a high degree of variation and inconsistency in the management of bulbar disease (1), presenting a critical need for the development of established practice guidelines to manage these patients. The NEALS Bulbar Subcommittee subsequently organized a bulbar protocol symposium to actively address these concerns and establish an operative set of bulbar recommendations to implement within our ALS clinics.

The NEALS subcommittee began development of a minimally invasive and time-efficient standardized assessment protocol for speech and swallowing in ALS, resulting in multiple summary recommendations. The Speech section focused upon eliminating other non-ALS causes during the initial evaluation, additionally establishing supplementary information to include FVC, ALS-FRS-R, PBA, dysphagia, medications and cognition. Summary recommendations included both speech and AAC referral at the time of the initial visit. This evaluation emphasized the basic elements of speech assessment including the oral motor exam, CNS-BFS, maximum sustained phonation and speaking rate. The evaluation of dysarthria comprised the subsystem involvement of respiration, phonation, resonance and articulation.

The swallowing section stressed a working pathway to guide clinical management of swallowing in dysphagia. When to arrange a speech language pathologist (SLP) referral and the timing of any invasive intervention were identified as key problems, best determined by assessing the neurologic status and rate of disease progression. The SLP evaluation was decided upon to include the following domains: diet, swallow safety, airway defense/physiologic capacity, self reported swallow measures, oral function, and pulmonary function. Specific objective testing and outcome recommendations were established for each of the above domains. Practice parameters focused on patient education regarding the roles of feeding tubes, oral hygiene, compensatory swallow strategies, dietary modifications, pulmonary hygiene with airway clearance, and basic life saving techniques. Open questions were raised concerning the use of videofluoscopy, monitoring diet progression with FOIS, swallow safety screening, peak cough flow, EAT-10 impact, respiratory measures, and basic oral mechanical testing of tongue, palate and lips.

The working subcommittee has established a clinical bulbar protocol to be instituted within ALS clinics and subsequently expanded to identify a best practice set of bulbar ALS guidelines, incorporating both international collaboration and individual systematic reviews of each of the agreed upon recommendations.

Acknowledgements: Logistical and technical support provided by NEALS/MGH. Funding provided by Cytokinetics.

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C73 The Oral Secretion Scale (OSS) predicts tolerance of non-invasive ventilation (NIV), the need for hospice or transition to tracheostomy ventilation (TV) and prognostic factors for survival in patients with ALS/MND

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Background: Non-invasive ventilation (NIV) can be tolerated in patients with ALS/MND, unless oral secretions become severe. An Oral Secretion Scale (OSS), a clinimetric scale was developed and validated (1,2) to measure secretions in correlation with swallow ability, quantifying the ability to clear secretions from airway: [4 = normal automatic swallow, no excessive secretions; 3 = automatic swallow decreased, minimal drooling, no pooling; 2 = conscious swallow required, moderate drooling, occasional pooling; oropharyngeal suctioning >5–30 ccs = 2–12/day; 1 = conscious swallow difficult, frequent drooling/pooling, oropharyngeal-suctioning >15–30 ccs = 13/24/day; and 0 = conscious swallow impossible, constant drooling/pooling, oropharyngeal-suctioning >15–60 ccs = >25/day] based on set of ALS/MND patients followed at home.

Objective: To assess the OSS for predicting tolerance of NIV, the need for hospice or transition to tracheostomy (TV) and prognostic factors for survival in patients with ALS/MND.

Methods: In this longitudinal, observational study, retrospective analysis of a prospectively collected cohort of 159 ALS/MND patients, followed from NIV initiation until death or start of TV during ongoing home visits, was performed. Survival curves from NIV initiation were calculated by Kaplan-Meier analysis. \( p-values = < 0.05 \) were considered significant. Frequency (%) of OSS scores when NIV became intolerable was determined. Univariate and multivariate Cox-regression analyses were used to determine factors affecting survival from NIV initiation.

Results: At NIV initiation, 87/159 (55%) patients had no impairment (OSS = 4). 22/159 tried NIV and failed during emergency hospitalizations. Of 137/159 patients who continued NIV, median months of survival from NIV initiation: 1.3 (95% CI 1.1–1.5), 4.6 (3.1–6.1), 10.7 (7.3–14.0) stratified by OSS scores of 1, 2–3, and 4, respectively; and 20.9 (95% CI 8.6–33.2), 7.5 (3.4–11.5), 6.2 (4.2–8.2), 2.1 (1.5–2.7) stratified by 24, 17–23, 4–16 and <4 h/d NIV used respectively. Survival was significantly \( (p = 0.0001) \) longer in patients with OSS score 4 than 1 at NIV initiation; and significantly \( (p < 0.0001) \) longer in patients who used NIV 24 h/d than <24 h/d. Of the patients who could no longer tolerate NIV, more than 80% had OSS scores of 1 or 0. Univariate and multivariate analyses (HR = 0.93, 95% CI 0.91–0.96, \( p = 0.0001 \)) and (HR = 0.98, 95% CI 0.90–0.95, \( p = <0.001 \)) respectively showed hours/day NIV used were a significant prognostic factor for survival.

Conclusions: OSS = 4 score patients can tolerate continuous NIV use and survive significantly longer than <OSS = 4 score patients. OSS score 1 reliably signals the inability to maintain effective upper airway clearance non-invasively, NIV intolerance, and the need for hospice or transition to TV. Survival correlates with NIV tolerance and hours/day NIV are used.

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C74 Trial of resistance and endurance exercise in amyotrophic lateral sclerosis

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Keywords: exercise, standard of care, clinical trial

Background: Among the first questions asked by patients with a new diagnosis of ALS often include: “Does exercise help slow the progression of the disease?”, “Is there any harm in exercising?”, or “What type of exercise is most appropriate for ALS patients?” However, there is a paucity of answers for people who suffer from an illness that affects their strength above all else. A randomized, controlled, large study evaluating the potential benefits of resistance and endurance exercise in ALS has not been systematically undertaken. In the American Academy of Neurology Practice Parameter for ALS, no recommendations were made regarding specific types of physical exercise in ALS management. Therefore, there is no consensus on the possible benefits, or hazards, of exercise formulated for ALS. In light of these observations, individuals with ALS were asked to participate in a randomized, 6-month, parallel group study which included exercise in one of three ways: weightlifting (resistance exercise), stationary bicycling (endurance exercise), and stretching/range of motion exercise (SROM) (the exercise regimen prescribed for most ALS patients).

Objective: Evaluate the safety and tolerability of resistance and endurance exercise in ALS participants as measured by their ability to complete this 6-month study.

Methods: Participants were randomized to Resistance, Endurance, or Stretching/Range of Motion (SROM—the exercise regimen prescribed for most ALS patients) exercises. All exercises were performed at home and under the direction of a physical therapist trained in ALS management. Primary outcome measures were tolerability of the exercises at 24 weeks defined by at least 50% of participants completing at least 50% of the prescribed exercise regimen. Secondary outcome measures included the ALSFRS-R, pulmonary FVC, and other measures of ALS function.

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**Results:** At 12 weeks, all three of the exercise regimens were well tolerated. At 24 weeks SROM and resistance exercises were well tolerated, with endurance exercise less well tolerated. All 3 forms of exercise were considered safe as there were no differences in the rates of disease progression among groups. There were no differences in the secondary outcome measures and feasibility for evaluating these measures was successful. In a *post-hoc* analysis, there was a trend towards fewer falls in the resistance and endurance groups.

**Conclusions:** This study demonstrates that SROM, resistance, and endurance exercise are all safe to be performed at the specified regimen without any worsening of outcomes as related to ALS function. Resistance and SROM exercises were the best tolerated over the 24-week period. The results of this study lay the groundwork for recommendations to patients regarding the type, frequency, intensity, and safety of specific exercise regimens in ALS care.

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**C75 The 100 collars project: a multi-centre evaluation of the Head Up cervical orthosis**

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**Keywords:** clinical care, clinical management

**Background:** People with amyotrophic lateral sclerosis (ALS) can experience weakness of the neck muscles with consequent head drop. This can be associated with pain, social embarrassment, and difficulties with communication and swallowing. Standard practice is to use a cervical orthosis to compensate for the weakness. Many of the cervical orthoses available were designed for other conditions and either provide too little support or are too rigid. We conducted a user centred design project with people with ALS, engineers, designers and healthcare professionals that led to the design of a specific orthosis to meet the needs of people with ALS – the Head Up orthosis. Head Up is a cervical orthosis which has a significant amount of customisability. The amount of support can be varied in a task specific manner or with disease progression. We have previously conducted a small scale evaluation which enabled refinement of the final design. We sought to undertake a much wider evaluation to establish performance of Head Up in routine clinical practice and to obtain information on a final design (optimal sizing and number of supports) to ensure a cost effective final product.

**Aims and objectives:**
- To evaluate the usability and acceptability of Head Up to people with ALS and for healthcare professionals
- To further refine design

**Methods:** We conducted a multi-centre, non-blinded, single arm before and after cohort study to evaluate the Head Up orthosis in patients with ALS who had need of a cervical orthosis. After a baseline questionnaire of previous collar use, neurological and anatomical assessments participants were fitted with a Head Up to use at home for one month. Participants returned at one month to complete an end of study questionnaire and assessment. Descriptive statistics were used to summarise the questionnaire results and the Wilcoxon signed-rank test was used to compare the results between previous collars and Head Up. Healthcare professionals were also asked to complete an assessment on practical aspects of the fitting of Head Up.

**Results:** 100 patients were recruited from 10 centres in the UK and Ireland. 80% chose to keep Head Up and continue to use it in preference to other collars after the month trial period. Head Up scored significantly better (*p*<0.005) then previous collars used by patients in terms of satisfaction, level of support offered, residual head movement possible, appearance, lack of interference with eating and drinking. Head Up was harder to fit. No significant differences were seen in terms of perspiration, breathing or swallowing.

**Discussion:** There was a strong preference for Head Up reported by individuals with ALS and by healthcare practitioners. Novel data from the anatomical assessments of participants necks will lead to improved matching of Head Up sizes to individual need.

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**C76 Treatment for cramps in amyotrophic lateral sclerosis/motor neuron disease: an updated Cochrane review**

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**Keywords:** muscle cramp, treatment, review

**Objective:** To systematically assess the effect of interventions on muscle cramps as a primary or secondary endpoint in people with ALS/MND.

**Method:** On 13 December 2016 we searched the Cochrane Neuromuscular Specialised Register, CENTRAL, MEDLINE and Embase. We searched NHSEED, DARE, and HTA for information relevant to
the Discussion. We also checked the reference lists of articles and contacted authors of trials for further information. We searched clinical trials registries for ongoing trials. We included all RCTs and quasi-RCTs of oral medications in people with ALS which assessed cramps as a primary or secondary outcome measure. We also included trials using subcutaneous or intravenous medications or physical therapy.

**Results:** We identified 15 studies including 4092 participants. Two trials of mexiletine 300 mg/d assessed muscle cramps, one as a primary outcome measure and one as a secondary; both trials demonstrated a reduction in the number of muscle cramps and cramp severity. Only one of the negative trials, one of tetrahydrocannabinol (THC), assessed cramps as the primary endpoint. Twelve studies assessed cramps as a secondary endpoint. The medications comprised vitamin E, baclofen, riluzole, L-threonine, xaliproden, gabapentin, and memantine.

None of the 12 studies demonstrated a favourable effect for the treatment of cramps in ALS/MND, but many studies were underpowered to draw a definite conclusion and some listed cramps as an outcome measure but did not provide detailed data. A meta-analysis supported the beneficial effect of mexiletine on muscle cramps. A meta-analysis of two small studies showed a statistically non-significant result for the amino acid L-threonine for the treatment of cramps in ALS/MND. We identified no study using physical therapy as a therapeutic intervention for cramps.

**Discussion:** There is evidence to support the use of mexiletine 300 mg/d for the treatment of muscle cramps in ALS/MND. More and larger RCTs evaluating treatments for muscle cramps in ALS/MND are needed.

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Session 8C Neuroimaging

C77 Tracing disease progression in ALS: a multimodal longitudinal imaging study of structural brain involvement

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Keywords: imaging, disease progression, biomarker

Background: More knowledge on how neurodegeneration develops over time may help to understand the progressive nature that characterizes amyotrophic lateral sclerosis (ALS), and may help to identify therapy that slows or stops disease progression. Longitudinal imaging studies have the potential to provide an anatomical characterization of disease progression related to clinical phenotype.

Objective: To investigate cerebral changes in patients with ALS, using a longitudinal, multimodal approach in a large cohort of ALS patients.

Methods: In total, 292 ALS patients, including 24 with a C9orf72 repeat expansion and 156 healthy controls participated in the study. We assessed cortical thickness, subcortical volumes and white matter connectivity from high resolution T1-weighted and diffusion-weighted magnetic resonance images. Clinical and imaging follow-up data were available for 150 ALS patients and 72 healthy controls. A linear mixed-effects model was used to assess changes in structural brain measurements over time.

Results: Longitudinal effects in ALS patients were detected by progressive cortical atrophy of primary motor regions and frontotemporal regions, smaller basal ganglia volumes, ventricle enlargement ($p < 0.05$) and a reduced fractional anisotropy of connections related to the motor cortex. ALS patients with bulbar onset showed involvement of frontotemporal regions at baseline, whereas patients with spinal onset showed additional atrophy of similar cortical regions over time. Considerable decrease in fractional anisotropy at baseline and additional cortical thinning over time were detected particularly in patients with short disease duration from symptom onset and relatively higher progression rate. Brain involvement in patients with a C9orf72 repeat expansion was characterized by widespread cortical involvement at baseline and extensive loss of white matter integrity over time.

Conclusions: Neuroimaging is capable of capturing longitudinal cerebral changes related to ALS and provides a tool for investigating neurodegeneration in ALS in vivo.

The multimodal approach shows that detection of grey and white matter involvement and the extent of brain involvement is dependent on the modality applied and influenced by phenotypic heterogeneity, with relevance for clinical trial design: it might be helpful in tailoring study design to the patient group by selecting the appropriate imaging techniques as a biomarker to study disease progression and the potential effects of new therapeutic strategies in more detail.

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C78 Patterns of cortical atrophy in amyotrophic lateral sclerosis and implications on prognosis

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Keywords: neuro-imaging, prognosis, cortical atrophy

Background: It has now been recognised that in ALS, pathological process is not restricted to the motor cortex and the anterior horn cells but may also affect fronto-temporal cortex, hippocampus, thalamus, substantia nigra, spinocerebellar and sensory pathways. Up to 30% of patients with ALS have evidence of cognitive impairment. C9orf72 gene expansion offers the genetic link between ALS and fronto-temporal lobar degeneration. The presence of clinical evidence of fronto-temporal dementia in a patient with ALS is considered as a poor prognostic factor. The prognostic implications of cortical atrophy in different brain regions in ALS has, however, not been studied.

Objective: (1) To understand the patterns of cortical atrophy in ALS; (2) To study the prognostic value of cortical atrophy at diagnosis in ALS.

Methods: This is a retrospective, observational study consisting of a conveniently sampled cohort of 249 patients with ALS, diagnosed at the Sheffield MND Care and Research Centre. The global cortical atrophy (GCA) scale was used to quantify cerebral atrophy on the MRI brain scans. The relationship between cortical atrophy and overall survival is assessed using Kaplan-Meier survival analysis and Cox regression analyses.

Results: 249 patients were studied; 130 males (52.2%) and 119 females (47.8%). The mean age of onset was 61.5 years and the mean diagnostic delay was 15.2 months. The commonest site of onset was lower limb (93 cases)
with 79 individuals having bulbar-onset disease. The mean survival was 36 months from disease onset. Motor strip atrophy was present in 50% of the cases, 31.3% of them showing bilateral motor strip atrophy. Moderate-to-severe fronto-temporal atrophy was present in 41% of the cases. Brain stem degeneration was seen in 34.2% of the cases. Kaplan-Meier analysis demonstrated higher degree of cortical atrophy being associated with poorer prognosis. Cox regression analysis (adjusted for gender, El Escorial category, and diagnostic delay) also demonstrated increasing hazard with increasing atrophy. This was true for fronto-temporal and motor strip atrophy, while brainstem atrophy did not have a significant effect on prognosis.

**Conclusion:** Serial MRI brain scans, during the disease trajectory of ALS potentially offer an objective measure for assessing disease progression. This would be especially beneficial in interventional trials such as gene therapy and stem cell transplantation trials. It would be desirable to have a structured approach to identify a radiological signature of ALS adding more value to MRI brain scans in the diagnostic and prognostic work-up of ALS.

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**C79 Perfusion imaging signatures of pathological spread across TDP-43 proteinopathies**

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**Keywords:** TDP-43, neuroimaging, frontotemporal dementia

**Background:** TAR DNA-binding protein 43 (TDP-43) pathological inclusions can result in pure motor (ALS-motor), pure behavioural (bvFTD) or a combination of motor and behavioural (ALS-FTD) impairments. However, TDP-43 mediated degeneration propagates through the brain differentially across these syndromes. Cerebral blood flow (CBF) may provide an early marker of potential impending damage and/or compensatory alterations that precede evidence of TDP-43 associated gray matter (GM) loss.

**Objective:** We aimed at evaluating proxies for pathological pread in various TDP-43 proteinopathies using GM atrophy and CBF measurements.

**Methods:** We evaluated ALS-motor (n = 18) bvFTD with either a neuropathological diagnosis and/or a known genetic mutation associated with FTLD-TDP pathology (n = 13), ALS-FTD (n = 15) patients and healthy controls (n = 33) who completed T1-weighted and pseudo-continuous arterial spin labeling (pCASL) magnetic resonance imaging (MRI). Data were processed using dedicated pipelines in Advanced Normalization Tools (ANTs) to compute and compare cortical thickness (CT) (p<0.05 tfce corr.) and partial volume-corrected CBF (p<0.05 unc).

**Results:** Relative to controls, bvFTD patients showed marked atrophy of the prefrontal and temporal cortices, hypoperfusion encompassing orbitofrontal regions and hyperperfusion in primary motor areas. ALS-motor patients showed no significant GM atrophy but evident hypoperfusion encompassing primary motor regions and hyperperfusion in prefrontal and temporal regions. ALS-FTD cases exhibited widespread atrophy encompassing primary motor and frontotemporal regions, significant orbitofrontal hyperperfusion as well as distinct areas of increased and decreased perfusion in primary motor regions.

**Discussion and conclusions:** Hyperperfusion in motor regions suggests compensatory responses to incipient underlying pathology in bvFTD phenotypes. Hyperperfusion in motor areas in ALS-motor cases suggests that perfusion alterations can provide an early marker of pathological spread and impending decline even in diseases lacking significant brain structural damage. Significant structural reductions encompassing primary motor and extra-motor brain regions in ALS-FTD may reflect the higher histopathological burden associated with the co-occurrence of the two diseases. In conclusion, hypoperfusion was associated with clinically manifest regions of degeneration while hyperperfusion was observed in clinically silent areas, suggesting that perfusion changes may mark specific vulnerable brain hubs and inform on trajectories of neurodegeneration across TDP-43 proteinopathies.

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**C80 Spinal cord gray matter atrophy as MRI biomarker for ALS patients**

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C81 Hypothalamic structure alterations in presymptomatic and symptomatic ALS

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Keywords: presymptomatic ALS mutation carriers, hypothalamus, energy metabolism

Background: Hypermetabolism is an early feature of ALS, and decreasing body mass index (BMI) is a prognostic factor. The hypothalamus is critically involved in the control of energy metabolism, but direct evidence of hypothalamic alterations possibly prior to onset of disease-defining symptoms has not been investigated yet.

Objective: To study the morphology of the hypothalamus in the presymptomatic and symptomatic phase of ALS in association with functional measures.

Methods: High-resolution T1-weighted MRI data together with clinical and laboratory parameters from a large monocentric cohort comprising 270 symptomatic ALS patients, 32 presymptomatic ALS mutation carriers, and 116 matched healthy controls were included in the analysis. An optimized approach was used for the MRI-based semi-automatic delineation of the hypothalamus. The hypothalamic volume was corrected for age, gender, and intra-cranial volume using a linear regression model.
Results: Compared with controls, the hypothalamus was substantially atrophied in symptomatic ALS patients as indicated by significantly reduced volume (–22%, \( p<0.0001 \)). Remarkably, the atrophy of the hypothalamus was already present in presymptomatic ALS mutation carriers (–13%, \( p<0.0001 \)). The hypothalamic volume loss was significantly correlated with decreasing BMI (\( p=0.04 \)), but not with the ALS-FRS-R nor with the whole-brain volume. Moreover, the age at motor disease onset was significantly correlated with the hypothalamic volume (\( p=0.003 \)).

Conclusions: Structural alterations of the hypothalamus appear to be already present prior to disease-defining symptoms in ALS, suggesting a presymptomatic phase mainly associated with metabolic alterations. In addition, the hypothalamic atrophy is possibly predictive for the age of motor symptoms onset. These findings call for future studies on hypothalamic function in ALS.

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Session 9A Neuroinflammation and Glial Signalling

C82 Imaging glial activation in people with amyotrophic lateral sclerosis (ALS)

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Keywords: [¹¹C]-PBR28 PET, glial activation, PLS

Background: [¹¹C]-PBR28 is a second generation radioligand that binds to translocator protein (TSPO) expressed on activated glial cells. We have previously shown preliminary in vivo evidence of increased glial activation in the motor cortex (MC) in people with amyotrophic lateral sclerosis (ALS). Here, we confirm our initial finding, study the longitudinal changes, and characterize the gray-white matter distribution of glial activation in a large cohort of ALS and primary lateral sclerosis (PLS) participants.

Methods: 53 ALS, 11 PLS and 21 healthy control (HC) participants underwent simultaneous MR-PET imaging using [¹¹C]-PBR28. Demographics and clinical assessments including upper motor neuron burden (UMNB) and the revised ALS functional rating scale (ALSFRS-R) were collected. [¹¹C]-PBR28 uptake was quantified as standardized uptake value ratio (SUVR), and compared between groups using voxelwise, surface-based, and region of interest (ROI) analyses. Cortical thickness (CT), fractional anisotropy (FA) and SUVR were compared between the groups and correlated with the clinical measures. [¹¹C]-PBR28 uptake, FA, and CT were compared over six months in 10 ALS subjects who completed follow-up scans.

Results: Voxelwise, surface-based and ROI analyses revealed increased [¹¹C]-PBR28 uptake in the MC in ALS participants compared to HC (P_{FWE}<0.05, P_{unc}=0.05, P<0.05; respectively). The increase in [¹¹C]-PBR28 uptake co-localized and correlated with decreased CT \(r=-0.49\; (p<0.05)\) and reduced FA \(r=-0.15\; (p=0.05)\). Gliosis correlated with higher UMNB \(r=0.53\; (p<0.05)\) and lower fine motor domain of ALSFRS-R \(r=-0.33\; (p<0.05)\). There was no correlation between [¹¹C]-PBR28 uptake and disease duration, or rate of progression. The correlation of [¹¹C]-PBR28 uptake in the MC at baseline and six months was \(r=0.84\; (p=0.0022)\). [¹¹C]-PBR28 uptake was predominantly increased in the sub-cortical white matter of the MC in the PLS group (P_{FWE}<0.05).

Interpretation: [¹¹C]-PBR28 uptake is increased in the MC in people with ALS and PLS, co-localizes with structural abnormalities, and correlates with relevant clinical measures. [¹¹C]-PBR28 uptake is remarkably stable over 6 months despite ALS progression. [¹¹C]-PBR28 PET is a promising candidate molecular biomarker to measure the impact of experimental treatments on glial activation in future ALS clinical trials.

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C83 MicroRNAs secreted by C9orf72 patient-derived astrocytes contribute to impairment in axonal growth and cell death in vitro

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Keywords: C9orf72, microRNAs, extracellular vesicles

Introduction: Astrocytes from mouse models of amyotrophic lateral sclerosis (ALS) and human samples are responsible for motor neuron (MN) death both in vitro and in vivo. In vitro experiments have indicated that most of the factors inducing MN death are secreted (1). In one of our previous publications we have demonstrated that astrocytes differentiated from induced neural progenitors (iAstrocytes) derived from patients carrying C9orf72 mutations are toxic to MNs (2). Previous studies investigating ALS astrocyte toxicity mainly focused on the proteomic analysis of astrocyte conditioned medium. These have identified promising targets to protect MNs (1); however, they have failed in identifying the actual factor(s) responsible for astrocyte toxicity. These could be proteins and/or nucleic acids and could be secreted directly in the medium or via extracellular vesicles mediating intercellular communication.

Objectives: In this paper we aim to identify the toxic factors secreted through extracellular vesicles by C9orf72 patient-derived astrocytes and design targets for therapeutic intervention.
Methods: Patient and controls fibroblasts were differentiated into induced neural progenitor cells (iNPCs) and subsequently differentiated into iAstrocytes. Mouse stem cells expressing the green fluorescent protein (GFP) under the MN specific promoter HB9 were differentiated into MNs and FACS sorted for co-culture with human iAstrocytes. Cell death and axonal length and branching were assessed using the Opera Phenix (Perkin Elmer) high content imaging system. MicroRNA microarrays 4.0 were used for microRNA quantification.

Results: Our data indicate that RNA species secreted via extracellular vesicles (EVs) by C9orf72 patient-derived astrocytes are largely responsible for astrocyte-mediated MN death in vitro. We found that C9orf72 astrocytes secrete significantly less EVs than controls. Since >85% of RNA contained in these vesicles are microRNA species, we performed microRNA microarray analysis of EVs isolated from C9orf72 patient astrocytes compared to controls. The analysis revealed dysregulation of microRNAs promoting vesicle trafficking and axonal growth. We validated expression dysregulation in 2 groups of microRNAs involved in these 2 pathways via qPCR. We then selected the most robust and consistent hits and validated their functional importance in vitro in a co-culture system with C9orf72 patient astrocytes and healthy motor neurons. Restoring the levels of miRNA-602 and miRNA-499 in our co-cultures resulted in complete rescue in axonal growth in motor neurons cultured on C9orf72 patient astrocytes.

Conclusions: We have used patient-derived astrocytes to identify potential therapeutic targets for gene delivery approaches and we have identified 2 very promising targets for future in vivo studies.

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C84 Slowing disease progression in the SOD1 mouse model of ALS by blocking neuregulin-induced microglial activation

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Keywords: neuregulin1 antagonist, therapeutic target, disease progression

Background: ALS is a neurodegenerative disease that spreads up and down the spinal cord until patients succumb. At the present time there are no effective treatments to slow disease progression. We previously reported that neuregulin (NRG) receptors are activated on microglia in the ventral horn and corticospinal tracts of ALS patients and in SOD1 mice (1,2). We have developed a targeted NRG antagonist (HBD-S-H4) that is a humanized fusion protein between NRG’s heparin-binding domain and a decoy HER4 receptor. This fusion protein blocks microglial activation in vivo in a model of chronic pain.

Objectives: To determine how NRG1 modulates microglial activation and affects disease progression in the ALS-SOD1 mouse model.

Methods: We used two different methods to deliver HBD-S-H4. In one approach, we injected HBD-S-H4 weekly through an implanted intracerebroventricular (icv) cannula for 8 or more weeks. In an alternative approach, we generated triple transgenic mice to express HBD-S-H4 in the central nervous system (CNS) of SOD1 mice (GFAP-tTA:tetO-HBD-S-H4:SOD1*G93A). Body weight, disease onset and progression, animal survival as well as pathological changes were measured.

Results: Recombinant HBD-S-H4 directly delivered into the CNS through implanted icv cannulas showed no signs of toxicity and significantly inhibited NRG receptor activation on microglia resulting in reduced microglial activation and motor neuron loss. The treatment also resulted in a delay in disease onset and an increase in survival. The therapeutic effect was dose-dependent that varied as a function of genetic background in 2 different strains of SOD1 mice. As a complementary drug delivery approach, transgenic mice expressing HBD-S-H4 driven by an astrocytic promoter (GFAP) had slower disease progression in a dose dependent manner, based on the level of HBD-S-H4 expression.

Discussion and conclusions: We have identified a therapeutic target of NRG1 receptor activation on activated microglia in both ALS patients and the ALS-SOD1 mouse model. These studies provide mechanistic insights into how NRG signaling on microglia may lead to disease progression and demonstrate the utility of a humanized fusion protein that blocks NRG as a novel therapeutic for human ALS.

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The role of microglia in TDP-43 clearance and redistribution in the zebrafish spinal cord

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Keywords: TDP-43, zebrafish, progression

Background: TDP-43 has been identified as the main protein of pathological inclusions that characterise ALS and FTLD (1,2). The clinical progression of ALS suggests a pathological spread of neurodegeneration through the nervous system (3,4). Strategies to prevent intracellular TDP-43 accumulation enhance its clearance and target the spread of neurodegeneration are of intense therapeutic interest.

Objectives: The goal of this project was to evaluate whether microglia actively phagocytose TDP-43 originating from dying motor neurons and whether this microglial phagocytosis of TDP-43 may be neuro-protective. We therefore examined in vivo in the zebrafish spinal cord the redistribution and release of TDP-43 in the presence and absence of microglia.

Methods: We have expressed ALS aggregates in single motor neurons by injecting zebrafish embryos with DNA constructs of fluorescent hTDP-43. Using UV laser ablation we selectively killed the expressing neurons and observed the fate of TDP-43 and response of microglia in real time at a single-cell level (5,6). Antisense morpholino injections of pu1 (spi1b) allowed us to effectively deplete the macrophage population during this process (7,8).

Results: Following UV-induced neuronal stress, we observed a stereotypical pattern of neurodegeneration which results in cellular rupture and dispersal of TDP-43. However, when microglia are absent, UV-stressed neurons undergo a prolonged and stereotypical neurodegeneration, characterized by cytoplasmic mislocalisation and dispersal of TDP-43. Further, on rare occasions following UV-induced neuronal death, we observe some TDP-43 accumulation in surrounding tissue.

Discussion and conclusions: Our data provide novel insights into the role of TDP-43 in the pathogenesis of ALS and emphasize the protective role of microglia during neurodegeneration. It suggests that forced degeneration of an individual neuron and the ensuing microglial response fit well with the early stages of disease progression in transgenic mice (9), and that disruption of microglial uptake can lead to abnormal dispersal of TDP-43 into neighbouring tissue. Thus, cytoplasmic TDP-43 accumulation in post-mortem ALS patient tissue might indicate that microglial phagocytosis is deficient or unable to cope with the degree of neuronal death that occurs during disease progression.

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C86 Hypermetabolism is associated with lower motor neuron burden, functional decline and predicts survival in ALS

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Keywords: prospective case-control study, hypermetabolism, survival

Background: Impairments in whole body metabolic homeostasis have been reported in ALS. The origin of hypermetabolism and its relation to clinical features of disease are unclear.

Objective: To investigate the prevalence of hypermetabolism in ALS in a prospective case-control study, and the relationship of hypermetabolism to clinical features of disease (the burden of upper and lower motor neuron signs) and to survival.

Methods: We studied 53 patients with clinically definite or clinically probable ALS and 57 control participants who were matched for age and gender. Predicted energy expenditure was determined relative to body composition, which was assessed by whole body air displacement plethysmography (BodPod, Cosmed). Measured energy expenditure was determined by indirect calorimetry (Quark RMR, Cosmed), and expressed as a percentage of predicted energy expenditure to obtain a metabolic index (MI). MI >120% was defined as hypermetabolism. Logistic regression was used to determine the difference in prevalence of hypermetabolism between cases and controls. Confounding was accounted for by inverse probability weighting. Linear mixed models were used to explore the longitudinal relationship between MI and clinical features. The differences between Kaplan-Meier curves between normometabolic vs. hypermetabolic ALS patients were compared using the log-rank test.

Results: There was an increased prevalence of hypermetabolism in ALS patients relative to controls (42% vs. 13%, adjusted odds ratio = 6.0; p<0.01). Hypermetabolism was associated with a greater lower motor neuron burden (p<0.01). We observed a greater change in ALSFRS-R in hypermetabolic individuals (p<0.01; –0.35 vs. –0.78 points/month). MI was inversely associated with survival; 18-month survival in normometabolic vs. hypermetabolic individuals was 93.8% vs. 20.9% (p<0.01).

Discussion and conclusion: This is the first study to establish the prevalence of hypermetabolism in ALS relative to fat free mass, the major determinant of energy expenditure (2). Higher MI in ALS patients who have significant lower motor neuron burden suggests that hypermetabolism may be linked with the loss of motor units, and supports the notion that hypermetabolism may originate from gross changes in skeletal muscle metabolism (3). In this study we show that hypermetabolic ALS patients have a faster rate of functional decline and lower survival, indicating important prognostic properties. This could be useful for stratifying patients for clinical trials. Patients with hypermetabolism might benefit from compensatory interventions.

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C87 Lipid metabolism and survival across the amyotrophic lateral sclerosis-frontotemporal dementia spectrum

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Keywords: frontotemporal dementia, lipid metabolism, dietary advice

Objective: Patients with amyotrophic lateral sclerosis and frontotemporal dementia exhibit prominent changes in eating behaviour that could potentially affect lipid levels. The present study aimed to document changes in lipid metabolism (cholesterol and triglycerides) across the ALS-FTD spectrum to identify potential relationships to eating behaviour, body mass index and effect on survival.

Methods: 128 patients were recruited for the study, incorporating 37 ALS patients, 15 ALS patients with cognitive and behavioural change (ALS-Plus), and 13 ALS-FTD, 31 behavioural variant FTD, with results compared to 31 healthy controls. Fasting total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL) cholesterol and triglyceride levels were measured and correlated to eating behaviour, (Appetite and eating health questionnaire), total caloric intake, reported fat intake, body mass index; effect on survival was examined using Cox regression analyses.

Results: There was a spectrum of lipid changes across the spectrum from ALS to FTD with increased triglyceride ($p<0.001$), and total cholesterol/HDL ratio ($p<0.001$), and lower HDL cholesterol levels ($p=0.001$) in ALS, ALS-Plus, ALS-FTD and bvFTD patients compared to controls. While there was no increase in total cholesterol levels, a higher cholesterol level was found to correlate with 5.5 times improved survival across the spectrum ($p=0.031$), triglyceride and HDL cholesterol correlated with BMI, but also with reported fat intake, cognition (Addenbrooke’s cognitive examination), disease duration and disease progression.

Conclusions: A spectrum of changes in lipid metabolism has been identified in ALS-FTD, with total cholesterol levels found to potentially impact on survival. These changes were partially mediated by changes in fat intake and BMI, but also may be mediated by the neurodegenerative process, offering the potential to modify these factors and slow disease progression.

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C88 Clinical characteristics and associated factors in amyotrophic lateral sclerosis patients with longer survival

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Keywords: biomarkers, HbA1c, creatine kinase

Background: Better understanding of survival factors in ALS could help physicians and patients to schedule therapeutic interventions. We conducted this study to evaluate the predictive factors associated with longer survival in ALS patients.

Methods: A total of 553 ALS patients were enrolled and divided into two groups: a training set (387 patients) and a validation set (166 patients). Disease-related features, personal history, comorbidities, hematologic tests and treatments were included as affecting factors to compare the ALS patients with survival time of beyond three years and less than three years. Risk factors for survival were identified using logistic regression analysis, and a nomogram created by R program was performed to predict the probability of longer survival in the training set, then receiver operating characteristic (ROC) analysis was applied to assess the predictive value of the nomogram model in the validation set.

Results: The median of survival time was 3.2 years for all patients. The survival time of beyond three years in patients was significantly associated with age, ALSFRS-R score, disease duration, disease delay, disease progression, HbA1c, BMI, hypoproteinemia, and NIPPV. Multivariate analyses revealed that age, rate of disease progression, HbA1c level, BMI, creatinine, CK, and NIPPV were independent predictors of longer survival. The ROC curve of the nomogram demonstrated good discrimination ability with the AUC of 0.915 (95% CI 0.789–0.923) in the validation set.

Conclusions: In ALS, serum CK, creatinine and HbA1c levels at baseline were independent biomarkers of longer survival, after correction for other known factors. The nomogram proposed an effective way to predict the probability of longer survival, and can help doctors to evaluate the disease progression and give personalized treatment.

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C89 Biomarker mixtures predict ALSFRS-R at time of diagnosis

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Keywords: biomarkers, ALSFRS-R, oxidative stress

Background: Previous studies have investigated whether baseline measures of oxidative stress related biomarkers predict ALSFRS function at baseline. A fundamental problem with this approach is the correlation between biomarkers, making it difficult to parse out which are important. The recent development of new methods to analyze highly correlated data has not been applied to the issue of biomarker prediction of ALSFRS.
Objective: Here we apply one new method, weighted quantile sums, to evaluate whether biomarkers associated with oxidative stress predict ALSFRS-R at baseline. The benefit of weighted quantile sums (WQS), compared to other methods, is that the associations between components of the WQS index and the outcome are taken into account.

Methods: Data were derived from COSMOS, a multicenter study of ALS patients recruited within 18 months of symptom onset and followed for 24 months or until death. A total of 355 patients were recruited at 16 study sites. At the baseline visit, blood and urine samples were obtained, processed, and stored until biomarker analyses. For this analysis, we were interested in biomarkers potentially related to oxidative stress: serum creatinine, serum uric acid, urinary 8-isoprostane, and urinary 8-Oxo-deoxyguanosine. We derived a biomarker index using weighted quantile sums; 30% of the sample was used in a ‘discovery’ analysis and 70% in a ‘validation’ analysis. The index was used as the primary exposure in a multiple regression model with ALSFRS-R as the dependent variable, and controlling for sex, race, age, ethnicity, duration of symptoms prior to the baseline visit, and site of symptom onset (spinal vs. bulbar).

Results: The derived index put 50% of the weight on urinary 8-Oxo-deoxyguanosine, 35% on serum uric acid, 12% on serum creatinine and 4% on 8-isoprostane. In the final regression analysis, each unit increase in the index was associated with a 0.45 point decrease (95% confidence interval –0.93 to 0.02) in ALSFRS-R at baseline (p = 0.06). This association appeared to be linear, as the inclusion of a quadratic term for the index was not statistically significant. In a sensitivity analysis in which we used 40% of the sample as discovery and 60% as validation, similar results were found.

Discussion: We find associations between an index of biomarkers and ALSFRS-R within 18 months of symptom onset. As baseline ALSFRS-R is highly predictive of disease progression and survival; these results indicate that baseline levels of oxidative stress may be important for prognosis.

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C90 Blood vitamin D levels correlate with amyotrophic lateral sclerosis severity: a prospective study

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Keywords: vitamin D, prognostic factor, severity score

Background: Some biological factors have been described as disease-modifiers in ALS, such as blood levels of cholesterol, creatinine, ferritin or vitamin D (VD). We previously showed that: 1) ALS patients with the lowest blood levels of VD had the worst prognosis; 2) VD improved motor neuron survival in culture; 3) VD completely blocked Fas-ligand-induced cell death. Contrasting results were published showing that ALS patients with the highest VD levels had the worst prognosis. Others did not find any relationship between VD levels and ALS prognosis. However, these studies raised several methodological concerns that needed to be addressed, all the more so as low VD blood levels have been implicated as a prognostic factor in a series of neurological disorders and this had been reinforced by cell culture as well as animal models.

Objectives: To prospectively study the clinical and prognosis profile of ALS patients according to VD levels, with statistical adjustment to confounding factors using multivariate analysis.

Methods: Patients were prospectively included if they had ALS for less than three years. The following data were systematically collected at inclusion: gender, age of onset, ALS duration, site of onset, slow vital capacity (SVC), weight (at inclusion and at ALS onset), ALSFRS-R score, blood VD levels. During patients’ follow-up SVC, weight and ALSFRS-R score were obtained quarterly. At the end of follow-up, mostly 1 year, weight loss, SVC loss and ALS severity score (ASS, mean number of points lost each month for ALSFRS-R scale) were calculated.

Results: 127 patients were included. Linear regression with a step-by-step selection of variables showed, in univariate analysis, that ASS significantly correlated with VD levels, ALS duration and SVC at inclusion, SVC and weight loss. When those significant variables were introduced in a multivariate model it was shown that ASS best correlated with blood VD levels, r = -0.0253, p<0.0001. Those with the highest blood VD levels had a less severe worsening of ALS. When categorizing VD levels (normal level >30ng/ml, deficiency <30 and >15, severe deficiency <15), to compare ASS between these three groups, statistical analysis showed that patients with the highest ASS were those with severe VD deficiency with a 60% more rapid course of ALS as measured by the worsening of ALSFRS-R scale score.

Discussion: This prospective study in ALS patients confirms our previous, retrospective, results: ASS, as measured by points lost at the ALSFRS-R scale each month, an important prognostic factor in the disease, was higher in patients with severe VD deficiency. Confounding factors, such as weight loss, may explain discrepancies between previous studies. The mechanisms implicated in the influence of VD in the disease could be at the lymphocyte or the neuronal level or both. We are now investigating these aspects.

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C91 Analyzing biological networks to identify novel disease pathways

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Keywords: systems biology, network modelling, integrative omics

Rapid advances in high-throughput technologies, including next-generation sequencing, proteomics, and metabolomics, are providing exceptionally detailed descriptions of the molecular changes that occur in diseases. However, it has been difficult to use these data to discover new therapeutic insights. Despite their power, each of these methods still only captures a small fraction of the cellular response. Moreover, when different assays are applied to the same problem, they provide apparently conflicting answers. I will show how specific network modeling approaches reveal the underlying consistency of the data linking the disparate observations. These patient-specific networks may provide critical insights for targeted therapies. Currently, we are applying these approaches to a range of diseases. I will outline how we are using these methods in a large, multi-center study of ALS patients and controls, and present some preliminary findings.

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C92 Early analyses of clinical and iPS motor neuron multi-omics signature from a large population of sporadic and familial ALS patients reveals verifiable subgroups and molecular pathways

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Keywords: iPS models, sporadic, multi-omics

Amyotrophic lateral sclerosis (ALS) and ALS/frontotemporal dementia (FTD) is a collection of different subtypes of patient populations and molecular etiologies, none of whom can be currently differentiated by clinical exam or by molecular profiling. Answer ALS was conceived and organized as a comprehensive multi-omics approach to ALS to ascertain, at a population level, the various clinical-molecular- biochemical subtypes of ALS. The overall organization was built on the collaborative NIH initiated NeuroLinc’s consortium which was started with a small collection control (n = 5) and ALS iPS cell lines including patients with sporadic ALS (n = 5) as well as mutant SOD1 (n = 5) and C9orf72 (n = 5) mutations.

18 months ago, the large national program known as Answer ALS was initiated to identify and follow 1000 ALS and ALS/FTD patients nationwide along with a cohort of matched controls patients. iPS motor neurons were blood-derived from each patient and these cells underwent multi-omic analyses including: whole genome sequencing, RNA transcriptomics, ATAC-Seq, proteomics, metabolomics, lipomics, high content imaging and longitudinal high-throughput single cell analysis. In addition, a smartphone based home monitoring system was employed to collect deep clinical data including fine motor activity, speech, breathing and linguistics/cognition. HIPPA compliant cloud databases were employed to store all data. Open access to early raw data is being instituted as well.

At the time of writing, more than 470 ALS patients have been enrolled and more than 200 iPS cell lines have already been generated from these patients and more than 300 whole genomes sequenced. Integrated clinical and biological signatures are now being generated using bioinformatics, statistics and computational biology to establish patterns that may lead to a better understanding of the underlying mechanisms of disease. Early multi-omics analysis of a trial subset of >30 sporadic ALS, ~10 C9orf72, mutant SOD1 and control iPS motor neuron cell lines were used to determine if biological subgroups could be identified. Definite subgrouping was readily identified even in this small subset. For example, C9 patients were found have prominent defects in nuclear transport, chromatin remodeling and RNA metabolism as fundamentally altered pathways with candidate pathway modulating drugs identified. For some subgroups, antisense oligonucleotides targeting relevant pathways could mitigate molecular injury – reverting cells towards control patient profiles. Relevant pathways and molecular targets are being verified in post-mortem brain tissue as well as fly models. A web portal for open source sharing of all data is being developed for widespread community based data analytics.
These studies demonstrate distinct reliably identifiable subgroups among the sporadic and familial patients and the great utility in iPS based approaches to disease pathophysiology and therapy discovery.

**C93 Molecular phenotyping of human neurons with TDP-43 pathology reveals derepression of transposable elements**

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*Keywords:* TDP-43, transcriptome, chromatin

**Background:** TDP-43 pathology, the presence of cytoplasmic aggregates and nuclear clearance, is the strongest correlate to neurodegeneration. While it is presumed that aberrant TDP-43 activity leads to downstream molecular aberrations that cause neuron death, the full spectrum of molecular changes associated with TDP-43 pathology in human neurons is not well defined.

**Methods:** Using a novel fractionation method based on subcellular fractionation and fluorescence activated cell sorting, we have isolated pathologic neuronal nuclei versus adjacent uninvolved neuronal nuclei from human tissue for molecular phenotyping.

**Results:** RNA-sequencing of 14 paired libraries revealed widespread transcriptomic alterations including differential expression of 5576 transcripts, 1044 alternative splicing events, preferential dysregulation of intronic RNA segments, and aberrant autoregulation of TARDBP. Gene enrichment and network analyses revealed several transcriptional modules that are associated with TDP-43 pathology, including genes which regulate chromatin structure and sub-networks related to DNA damage and DNA repair. RNA-sequencing of TDP-43 transgenic mice showed similar changes, together with a loss of neuronal heterochromatin that was readily apparent histologically. Based on these findings, we performed ATAC-seq on fractionated human neuronal nuclei to identify changes in chromatin accessibility associated with TDP-43 pathology. Bioinformatic analyses demonstrated that pathologic neurons exhibit increased accessibility of heterochromatic regions of the genome that were highly enriched for LINE-1 elements. Moreover, LINE-1 DNA content was significantly increased in neurons with TDP-43 pathology. Finally, TDP-43 overexpression and CRISPR-Cas9 based TARDBP knockout experiments in cultured cells confirm that abnormal TDP-43 protein expression alters LINE-1 retrotransposition activity.

**Conclusions:** These findings suggest that alterations in chromatin accessibility due to the presence of TDP-43 pathology may be associated with reactivation of transposable elements. Our results coincide with evidence from other independent groups supporting the possibility that transposable elements or endogenous retroviruses may be activated in the setting of ALS. Collectively, these results raise the possibility that pharmacologic inhibition of retrotransponson activity may mitigate the neurotoxic effects of TDP-43 pathology.

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**C94 Extensive RNA sequencing study in brain tissue obtained from patients harboring a C9orf72 repeat expansion**

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*Keywords:* C9orf72, RNA sequencing, transcriptome

**Background:** This study focuses on patients who received a pathological diagnosis of frontotemporal lobar degeneration (FTLD) and motor neuron disease (MND) due to a repeat expansion in chromosome 9 open reading frame 72 (C9orf72), which represents the most frequent genetic cause of these fatal neurodegenerative diseases.

**Objective:** Next-generation sequencing (RNAseq), followed by in-depth bioinformatics and statistical analysis, was performed to discover pathomechanisms specific to C9orf72-related diseases.

**Methods:** In total, we selected 34 C9orf72 expansion carriers with a pathological diagnosis of FTLD in the presence or absence of MND, 44 patients with a pathological diagnosis of FTLD in the presence or absence of MND without known mutations, and 24 control subjects without neurological diseases. High-quality RNA was extracted from frontal cortex tissue stored at the Mayo Clinic Florida Brain Bank. RNAseq was performed by the Mayo Clinic Medical Genome Facility. After quality control steps, expression values were adjusted for age, gender, RNA integrity number (RIN), and flow cell. Weighted correlation network analysis (WGCNA) was performed to identify clusters (modules) of co-expressed segments, and aberrant autoregulation of TARDBP. Gene enrichment and network analyses revealed several transcriptional modules that are associated with TDP-43 pathology, including genes which regulate chromatin structure and sub-networks related to DNA damage and DNA repair. RNA-sequencing of 14 paired libraries revealed widespread transcriptomic alterations including differential expression of 5576 transcripts, 1044 alternative splicing events, preferential dysregulation of intronic RNA segments, and aberrant autoregulation of TARDBP. Gene enrichment and network analyses revealed several transcriptional modules that are associated with TDP-43 pathology, including genes which regulate chromatin structure and sub-networks related to DNA damage and DNA repair. RNA-sequencing of TDP-43 transgenic mice showed similar changes, together with a loss of neuronal heterochromatin that was readily apparent histologically. Based on these findings, we performed ATAC-seq on fractionated human neuronal nuclei to identify changes in chromatin accessibility associated with TDP-43 pathology. Bioinformatic analyses demonstrated that pathologic neurons exhibit increased accessibility of heterochromatic regions of the genome that were highly enriched for LINE-1 elements. Moreover, LINE-1 DNA content was significantly increased in neurons with TDP-43 pathology. Finally, TDP-43 overexpression and CRISPR-Cas9 based TARDBP knockout experiments in cultured cells confirm that abnormal TDP-43 protein expression alters LINE-1 retrotransposition activity.

**Conclusions:** These findings suggest that alterations in chromatin accessibility due to the presence of TDP-43 pathology may be associated with reactivation of transposable elements. Our results coincide with evidence from other independent groups supporting the possibility that transposable elements or endogenous retroviruses may be activated in the setting of ALS. Collectively, these results raise the possibility that pharmacologic inhibition of retrotransponson activity may mitigate the neurotoxic effects of TDP-43 pathology.

**Acknowledgements:** Supported by grants from the Judith & Jean Pape Adams Charitable Foundation, the Doris Duke Charitable Foundation, and the NIH (R01NS095793, R21NS097749, P30AG010124, P01AG017586, T32AG000255).
genes significantly enriched for gene ontology (GO) processes.

**Results:** When comparing C9orf72 expansion carriers to control subjects, we detected modules indicative of neuronal loss (eg synaptic transmission; Bonferroni corrected p-value \(p_c = 5.0e-49\)) and inflammatory processes (eg immune system process; \(p_c = 4.2e-25\)), confirming the validity of our approach. Additionally, we observed a significant enrichment for genes functioning in protein folding (eg response to unfolded protein; \(p_c = 1.9e-09\)) and the electron transport chain (eg respiratory electron transport chain; \(p_c = 2.7e-19\)). Subsequently, we compared C9orf72 expansion carriers to disease controls. In these patients, the presence of a C9orf72 repeat expansion seemed to affect several processes, such as protein glycosylation (eg protein N-linked glycosylation; \(p_c = 2.0e-06\)) as well as metabolic and catabolic processes (eg carboxylic acid metabolic process; \(p_c = 5.4e-21\)), which uncovered highly connected genes present within modules (hub genes).

**Discussion and conclusions:** Our transcriptomic assessment of a large cohort of patients carrying a repeat expansion in C9orf72 revealed general processes associated with neuronal loss and inflammation, but also other processes, including protein folding and glycosylation. As such, our findings help to increase our understanding of C9orf72-related diseases, and additionally, they point to hub genes that may represent promising biomarkers or therapeutic targets.

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**C95 Integrated molecular landscape of amyotrophic lateral sclerosis provides insights into disease etiology**

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**Keywords:** molecular landscape, estradiol, axonal functionality

**Background:** Multiple environmental and genetic risk factors contribute to amyotrophic lateral sclerosis (ALS), but the relationship between these risk factors and the biological mechanisms underlying ALS is largely unclear.

**Objectives:** Gain insight into the aetiology of ALS and identify novel putative drug targets for the disease.

**Methods:** We conducted genetic network and literature analyses of the top-ranked findings from 6 genome-wide association studies of sporadic ALS (involving 3589 cases and 8577 controls) as well as genes implicated in ALS aetiology through other evidence, including familial ALS candidate gene association studies. We integrated the functional interactions between the proteins encoded by these ALS-linked genes into a molecular landscape of ALS.

**Results:** The ALS landscape allowed the identification of 3 main biological processes that interact with each other and are crucial to maintain axonal functionality, especially of the long axons of motor neurons, ie 1) Rho-GTPase signalling; 2) signalling involving the 3 regulatory molecules estradiol, folate, and methionine; and 3) ribonucleoprotein granule functioning and axonal transport. Interestingly, oestradiol signalling is functionally involved in all 3 cascades and as such an important mediator of the landscape. Furthermore, epidemiological findings together with an analysis of possible gender effects in a cohort of sporadic ALS patients indicated that oestradiol may be a protective factor, especially for bulbar-onset ALS.

**Discussion and conclusions:** Therefore, our molecular landscape of ALS suggests that abnormalities within three interconnected molecular processes involved in the functioning and maintenance of motor neuron axons are important in the aetiology of ALS. Moreover, oestradiol appears to be an important modulator of the ALS landscape, providing important clues for the development of novel disease-modifying treatments.

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Session 10B Cognitive Change

C96 The ALS-FTD continuum

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Keywords: frontotemporal dementia, genetics, cognitive change

Since the discovery of C9orf72 in 2011, the field of ALS-FTD has exploded (1). Mouse models of C9orf72 allow development of potential therapies to impact both ALS and FTD. A newly discovered gene, TANK binding kinase-1 (TBK-1) is also changing our thinking about ALS and FTD. Genetic testing is expanding to include sporadic ALS and FTD while our definition of 'familial ALS' is rapidly changing. Neuroimaging advances allow detection of different forms of ALS-FTD to further characterize patients while advances in identifying biomarkers may soon allow a diagnosis of ALS and/or FTD before a clinical diagnosis can be made.

ALS and FTD share a common pathology as well, Transactive Response DNA-binding Protein 43 (TDP-43). In addition, chronic traumatic encephalopathy is associated with both ALS and FTD. Genetic testing is expanding to include sporadic ALS and FTD while our definition of 'familial ALS' is rapidly changing. Neuroimaging advances allow detection of different forms of ALS-FTD to further characterize patients while advances in identifying biomarkers may soon allow a diagnosis of ALS and/or FTD before a clinical diagnosis can be made.

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C97 A population-based study of cognition in the ALS-FTD spectrum: the incidence and nature of language changes

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Keywords: ALS-frontotemporal spectrum disorder, neuropsychology, language

Background: Cognitive and behavioural changes are now recognized as core aspects in amyotrophic lateral sclerosis (ALS). Previous population-based studies showed that 40% of incident patients present with cognitive impairment (1,2). However, these conclusions have been essentially drawn from neuropsychological batteries focusing on executive function. Recent findings suggest that the neuropsychological profile in ALS extends beyond executive dysfunction, involving changes in language, social cognition and behaviour. This heterogeneous profile of frontotemporal nature, in conjunction with the understanding of ALS and FTD as overlapping disease phenotypes clinically, genetically and neuropathologically, have led to the consideration of ALS as a frontotemporal spectrum disorder (ALS-FTSD). In this context, revised diagnostic criteria for ALS-FTSD have been published (3), with emphasis on previously neglected cognitive functions such as language. Language changes in ALS have been described (4), although these have not been systematically studied in large population-based incident samples.

Objectives: To investigate the incidence and nature of language changes in a large population-based cohort of incident ALS patients.

Methods: This is a prospective population-based study of cognition in ALS. Each newly diagnosed ALS case attending the Irish National ALS Clinic since December 2014, and up to September 2017, is recruited (n≈120). Participants undergo comprehensive neuropsychological assessment, with a focus on language. Performance is compared to an age-, gender-, education- and premorbid IQ-matched healthy control sample (n = 100).

Results: Preliminary results on a subset of participants (non-demented ALS n = 55; healthy controls n = 50) indicated that language impairment was present in 19.6% of the patient sample, and it was significantly
more frequent than in an age-, education- and IQ-matched healthy control sample (4%; $p = 0.037$). While language deficits and executive dysfunction were associated, executive deficits only accounted for 47% of language impairment. The patient group performed significantly worse on measures of verb comprehension ($p = 0.004$), word spelling ($p = 0.018$), and grammatical comprehension ($p = 0.001$).

**Discussion and conclusions:** These results indicate the presence of language impairment in incident ALS cases, which are not fully accounted for by executive dysfunction. This outcome is to be confirmed on achievement of the full sample size, and will be presented and discussed relative to relevant published literature. The incidence and nature of neuropsychological deficits in this population-based incident sample considering the revised diagnostic criteria for ALS-FTSD will also be explored and presented. This work is a natural continuation of cognitive phenotyping in ALS, which helps identify disease markers and informs clinical and care management.

**Acknowledgements:** This work is funded by the Motor Neurone Disease Association, as part of a PhD investigation into language changes in the ALS-FTSD.

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**C98 Language is the cognitive function, which is most vulnerable to change in ALS**

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**Keywords:** language, longitudinal, cognition

**Introduction:** Up to 50% of ALS patients show cognitive decline and/or deficits in at least 1 single cognitive test. Longitudinal studies on cognition in ALS are still rare. Therefore, the aim of the present study was a cross-sectional and a longitudinal observation of changes in cognition and behaviour in a large clinical population of patients with ALS consecutively attending the ALS in- and out-patient clinic in Ulm, Germany. Data were compared to healthy controls by using the Edinburgh Cognitive and Behavioural Amyotrophic Lateral Sclerosis Screen (ECAS).

**Subjects and methods:** Overall, 611 patients diagnosed with ALS and 49 healthy controls were included. All subjects were assessed with the ECAS, addressing language, verbal fluency, executive functions, memory, visuospatial functions and behavioural changes. All healthy controls and 119 of the ALS patients were assessed twice in a time-span of minimum 5 months with the same questionnaires. Caregivers of 464 ALS patients filled out the questionnaire on behavioural and psychotic symptoms.

**Results:** Patients showed significant improvement in the ECAS total score ($p = 0.001$) and in the domains of fluency ($p < 0.001$) and memory ($p = 0.047$) in the course of 6 months but not in language, executive and visuospatial function. Healthy controls showed significant improvement in the ECAS total score ($p = 0.004$) and in the domains of language ($p = 0.002$) and memory ($p = 0.003$). Other domains showed no changes between the 2 assessments due to ceiling effect in healthy subjects. Overall, 33% of the patients presented with behavioural changes, mostly apathy and/or inertia. ALSFRS score was positively correlated with all cognitive domains (for all $p < 0.05$). Progression rate was negatively correlated with all domains ($p < 0.05$) apart from fluency ($p > 0.05$) and behavioural symptoms were negatively correlated with all domains ($p < 0.05$) apart from language ($p > 0.05$).

**Conclusion:** In the current study patients with ALS and healthy controls presented with improvement of performance in memory and fluency tasks being accounted for by a learning effect. In addition, healthy controls showed a significant improvement in language function which was not seen in patients, suggesting that patients exhibit reduced language skills over the course of 6 months.

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**C99 Behavioural changes in bvFTD and ALS-FTD: a prospective study**

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**Keywords:** frontotemporal dementia, cognitive, behaviour

**Background:** Behavioural changes are core features of amyotrophic lateral sclerosis with frontotemporal dementia (ALS-FTD) (1), yet few direct comparisons have been made of the behavioural profile in ALS-FTD and bvFTD alone. An earlier study suggested that there may be differences; however, this study was limited by its retrospective nature.

**Objectives:** To determine, in a prospective cohort, whether patients diagnosed with ALS-FTD show the
same pattern and degree of behavioural change as patients with bvFTD alone.

**Methods:** Detailed behavioural questionnaires and interviews were administered to carers of 20 bvFTD and 16 ALS-FTD patients, recruited from specialist dementia and MND centres in the north-west of England. Carer reports were rated against the 13 behavioural features described in current consensus criteria for bvFTD (2). Patients also completed detailed neuropsychological assessments.

**Results:** There were no significant differences between the groups in terms of age, gender or duration of illness. More behavioural features were endorsed for patients with bvFTD (Mean = 8.6, SD = 1.79) than with ALS-FTD (Mean = 5.7, SD = 2.77) t(34) = 3.81, p = 0.001. Chi square analysis showed that the bvFTD group was more likely to exhibit a loss of manners/decorum (p = 0.001), increased impulsivity (p = 0.014), reduced empathy (p = 0.049), motor stereotypes (p = 0.004) and complex repetitive behaviours (p = 0.042). By contrast, apathy, inertia and diminished social engagement as well as socially inappropriate behaviour and dietary changes were reported with comparable frequency in bvFTD and ALS-FTD. Executive dysfunction was common and did not differ between groups. (Fisher's exact test was used where expected cell counts were less than 5).

**Discussion and conclusions:** The ALS-FTD classification is frequently taken to imply that the behavioural characteristics are identical to those of bvFTD patients without ALS. This prospective study suggests that this may not be so. Even in a relatively small consecutive cohort of 20 bvFTD and 16 ALS-FTD patients there are detectable differences in behaviour. ALS-FTD appears to be most commonly characterised by apathy-related behaviours combined with executive dysfunction. Frank signs of disinhibition, impulsivity and stereotyped behaviours are less common than in bvFTD. The finding of qualitative differences in behavioural profile suggests that ALS-FTD and bvFTD may represent distinct clinical phenotypes.

**Acknowledgements:** This work forms part of a PhD studentship funded by the Motor Neurone Disease Association.

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**C100 Longitudinal assessment in ALS using the Edinburgh Cognitive and Behavioural ALS Screen (ECAS)**

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**Keywords:** ECAS, cognitive longitudinal assessment, practice effect

**Background:** Even if cognitive-behavioural involvement in amyotrophic lateral sclerosis (ALS) is now well-established, the longitudinal evolution of such features is a less investigated domain, due to the lack of adaptation to verbal–motor disabilities.

**Objectives:** We present clinical data about cognitive performances of ALS patients at the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) on repeated longitudinal testing. Moreover, we aim to assess if the ECAS could detect cognitive-behavioural changes over time.

**Methods:** 168 ALS patients (114 males, 54 females) were recruited. All patients from the validation study (baseline – V0) were invited to take part in a longitudinal study of cognitive changes in ALS, with follow-up at 6 (V1), 12 (V2) and 24 (V3) months, when possible due to patients’ clinical condition. The ECAS was administered, together with standard cognitive screening tools (Frontal Assessment Battery - FAB; Montreal Cognitive Assessment - MoCA) and psychological questionnaire for anxiety and depression.

**Results:** 168 ALS patients performed the ECAS at V0; 48 patients performed the exact same version of the ECAS after 6 months (V1). 20 patients were able to perform the ECAS again after 12 months (V2) and a small proportion...
of patients (n = 5) were tested again after 24 months (V3). No significant differences were found in patients’ performance between V0 and V1, neither in any ECAS subdomain and ECAS Total nor in FAB and MoCA. On the contrary, patients showed a significant improvement in their performance between V0 and V2 at some scores of the ECAS; in particular, the ECAS Total Score and ALS-Specific subscores as well as subdomains of language, executive functions, and memory were significantly higher at V2 compared to baseline. All ALS patients were able to complete the ECAS without any difficulties. Even after 12–24 months, the ECAS was still feasible for 100% of our ALS patients.

Discussion and conclusions: Patients showed practice effects on repeat application of the same test version in almost all sections. Only domains testing fluency and visuospatial function, as well as ALS-Non Specific sub-score, FAB and MoCA, did not improve after six and 12 months. The lack of a practice effect for such tests could probably be due to a ceiling effect with high initial scores. Compared to non-specific cognitive measures (FAB, MoCA), the ECAS was shown to be more feasible for ALS patients, and fully administrable also in advanced stages of the disease; the presence of subtasks involving motor and verbal skills that accommodate for physical disability could probably be responsible for such findings. In conclusion, even if parallel forms of ECAS could enhance a better cognitive involvement detection over time, our results suggest the feasibility of the ECAS along the course of the disease.

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C101 Development and clinical implications of the brief Dimensional Apathy Scale (b-DAS)

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Keywords: apathy, Dimensional Apathy Scale, assessment

Background: Apathy is a prominent demotivational symptom in neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) (1) and Alzheimer’s disease (AD) (2). It is considered a multidimensional syndrome, composed of different subtypes which can be measured using the Dimensional Apathy Scale (DAS) (1,3), a 24-item tool assessing Executive, Emotional and Initiation apathy, independent of motor disability. Due to increasing diagnosis, awareness and impact of apathy, a concise yet comprehensive measure is needed in clinic. The aim was to reduce the DAS using a large mixed neurodegenerative disease sample (AD and ALS) to form the brief DAS (b-DAS).

Method: Data from 102 non-demented ALS patients and 102 AD patients of responses to the informant/carer DAS were utilised, with additional availability of informant/carer Apathy Evaluation Scale (AES) and the Geriatric Depression Scale-Short form (GDS-15), standard apathy and depression measures. Mokken analysis was performed on each DAS subscale (Executive, Emotional and Initiation) for initial item reduction based on discrimination (Hi). Item endorsement (mean item score) was also examined. Item-total correlational analysis was performed, with the AES total, to determine convergent validity, and the GDS-15 total, to determine divergent validity, to establish the final structure of the b-DAS.

Results: AD and ALS patients were well matched for years of education, but differed on gender distribution and age. However, there was no correlation with age and no gender differences on apathy or depression. Mokken analysis on each DAS subscale resulted in all 8 Executive and all 8 Initiation items being retained, with 3 Emotional items showing weakness in Hi (<0.3) and were consequently removed. Of the remaining items, those with a stronger positive correlation with the AES (r>0.5) and also a moderate to weaker correlation with the GDS-15 (r<0.5), as well as those theoretically coherent to each subscale, were selected. This resulted in the b-DAS composed of 9 items, equally weighted over the Executive, Emotional and Initiation subscales.

Discussion and conclusion: The b-DAS is a robust yet short multidimensional apathy instrument composed of 9 items that correlate with a gold-standard apathy measure (convergent validity), while reducing the association with depression (divergent validity). It is appropriate for use in the clinic and research to quickly and comprehensively screen for apathy subtype impairments in neurodegenerative disease, with implications for design of motivation-based interventions.

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C102 SOD1 mutations in ALS: lessons learned and the path forward

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Mutations in the gene encoding cytosolic superoxide dismutase (SOD1) were identified as causes of ALS in 1993. In the subsequent 25 years, this discovery has fuelled diverse investigations into the molecular biology of this disease. Approaches have spanned many technologies including studies of human tissues at autopsy and diverse transgenic and in vitro models (now including stem cell-derived cultures of motor neurons).

Several conclusions are suggested by these investigations: 1) Identification of a single, primary pathogenic event has been elusive. Rather, motor neuron degeneration in ALS entails several concurrent events impacting multiple cellular processes simultaneously. As a corollary, these processes are likely to differ in different subcellular compartments (dendrites, nucleus, cytosol, axon, neuromuscular junction); 2) The molecular pathophysiology initiated by mutant SOD1 overlaps with that evoked by many other ALS genes and is also evident in many instances of sporadic ALS. In turn, this has suggested the hypothesis that 3) non-mutational, post-translational disturbances in wild-type SOD1 may be pathogenic in sporadic ALS; 4) A critical element in SOD1 toxicity is conformational instability and misfolding, consistent with the view that mutant (and probably also wild-type) SOD1 can self-assemble to propagate adverse misfolding in a prion-like manner, both within and between cells; 5) Taken together, these observations imply that an important therapeutic option in SOD1-mediated ALS, and perhaps other types of ALS (including sporadic ALS), is inactivation of the mutant, cytotoxic protein. Several innovative approaches to silencing the SOD1 gene are under investigation, including small molecules that inhibit the SOD1 promoter, anti-sense oligonucleotides, synthetic microRNA delivered via adeno-associated virus, and most recently SOD1 gene editing.

Some of these methods have recently proven safe in pilot human trials, encouraging cautious optimism that meaningful treatments for SOD1-related ALS are attainable in the foreseeable future.

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