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# SESSION 1 JOINT OPENING SESSION

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# SESSION 1 JOINT OPENING SESSION

## C1 WHAT IS NEEDED TO ADVANCE A DRUG CANDIDATE INTO CLINIC TRIALS? THE PERSPECTIVE OF ONE BIOTECH COMPANY

SANDROCK A

*Biogen Idec, Inc, Cambridge, MA, USA*

*Email address for correspondence: alfred.sandrock@biogenidec.com*

*Keywords: clinical trials, disease-modifying drugs, biomarkers*

The discovery of the genetic causes of ALS has accelerated the pace of discovery of biological pathways and targets against which compelling disease-modifying drug candidates are emerging. What is needed to advance these compounds into clinical trials? The answer is complex and likely to vary widely depending on who is asked.

At Biogen Idec, the decision to transition a drug from Research to Development (R to D), ie, the decision to begin IND/CTA-enabling toxicology studies under GLP conditions is an important milestone. It signifies that a research program has matured to a stage that justifies the significant investment of human and financial resources required to establish safe-use conditions and to commence Phase 1 clinical trials. Elements that go into this important decision include:

1. Does the drug address an important unmet need? Where would the drug fit in the therapeutic landscape of the disease?
2. Is the mechanism of action known? Is the biological activity of the drug anticipated to inhibit a biological pathway that causes worsening of disease or activate important protective/restorative mechanisms? What is the evidence that the pathway is operative in humans?
3. What is the pharmacological activity of the drug in relevant *in vitro* assays and *in vivo* models? What is known about the potency of the drug and its specificity for the target/pathway of interest relative to other targets/pathways?
4. What is known about the pharmacokinetics and tissue distribution of the drug? Will it reach the cells of interest so as to engage its target and produce the necessary biological effects in the relevant tissues?
5. If the compound is a small molecule, what is known about its absorption, distribution, metabolism, and excretion? Have the parent compound and its active metabolites passed the battery of tests that are predictive of its safe use in humans? Are any drug-drug interactions anticipated, especially with drugs commonly used by the target population?
6. Is the dose/duration at which the drug is predicted to be required for the beneficial effect, safe enough to justify exposing humans to the risk of taking the drug? What is the 'safety margin'? What are the target organs of toxicity?
7. Can informative Phase 1 and Phase 2 clinical trials be done? What biomarkers are available that could be used in humans that would: a) enrich for patient cohorts likely to respond to the drug and/or avoid harm from the drug; b) estimate drug target engagement; c) measure the impact of the drug on the biological pathways that are thought to be necessary in order to achieve efficacy; d) measure the impact of the drug on biological pathways that could cause harmful effects, especially in the target organs of toxicity, and predict the effect of the drug on clinical endpoints?

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# SESSION 2A RNA PROCESSING AND DYSREGULATION

## C2 REPEAT ASSOCIATED NON-ATG (RAN) TRANSLATION IN NEURODEGENERATIVE DISEASE

ZU T[1], LIU Y[1], BANEZ-CORONEL M[1], CLEARY J[1], REID T[1], TRONCOSO J[2], RANUM L[1]

[1]*University of Florida, Gainesville, Florida, USA,* [2]*Johns Hopkins University, Baltimore, MD, USA*

*Email address for correspondence: ranum@ufl.edu*

*Keywords: RAN translation, RNA Foci, microsatellite expansion*

For a group of neurological diseases caused by microsatellite expansions, mutations within predicted coding or non-coding regions are thought to cause disease by protein or RNA mechanisms.

In 2011, we discovered that in the absence of an AUG initiation codon, expanded CAG repeats can express homopolymeric proteins from all three reading frames. We showed this repeat-associated non-ATG (RAN) translation is hairpin-dependent, occurs without RNA editing and is observed in cell culture, as well as spinocerebellar ataxia type 8 (SCA8) and myotonic dystrophy type 1 (DM1) tissues.

We now provide evidence that RAN translation is a general mechanism that occurs across a variety of disease-causing expansion motifs, including the C9ORF72 GGGGCC hexanucleotide-expansion mutation which causes amyotrophic lateral sclerosis /frontotemporal dementia (ALS/FTD).

In this study, we demonstrate that sense and antisense C9ORF72 expansion transcripts accumulate in both the nucleus and cytoplasm in patient tissues. Additionally we show that both sense and antisense C9ORF72 expansion mutations produce dipeptide expansion proteins with Gly-Ala, Gly-Pro, Gly-Arg, Pro-Arg, Pro-Ala expansion motifs. Cell culture studies show RAN translation of these repeats occurs with as few as 30 repeats and that these proteins are toxic.

In order to detect novel RAN proteins *in vivo,* we have generated several panels of antibodies and show that ALS/FTD-dipeptide proteins accumulate as protein aggregates in several regions in C9ORF72 positive, but not control autopsy brains. Furthermore in order to investigate the mechanisms of RAN translation, we have developed a novel BAC transgenic mouse model of the disease.

In summary, this investigation demo nstrates that the discovery of RAN translation has implications for understanding fundamental mechanisms of protein synthesis and translational control, and should now be considered for a broad category of neurological disorders.

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## C3 ANTISENSE AND SENSE RNA FOCI DERIVED FROM REPEAT EXPANSIONS OF C9ORF72 HAVE SIMILAR INTERACTIONS BUT DISTINCT EXPRESSION PATTERNS

COOPER-KNOCK J, HIGGINBOTTOM A, WALSH MJ, STOPFORD M, HIGHLEY RJ, INCE P, WHARTON S, KIRBY J, HAUTBERGUE GM, SHAW PJ

*University of Sheffield, Sheffield, UK*

*Email address for correspondence: j.cooper-knock@sheffield.ac.uk*

*Keywords: C9ORF72, immunohistochemistry, RNA*

**Background:** GGGGCC repeat expansion of C9ORF72 represents the most common genetic variant of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Currently the mechanism of pathogenesis is unknown but it is suggested that gain-of-function toxicity may be caused either directly, by RNA foci transcribed from the repeat sequence or indirectly, via their translation into dipeptide repeat protein. We and others have determined protein binding partners of these RNA foci. It has been shown that RNA foci are formed by both sense and antisense transcription; we aim to determine whether the location and behaviour of these species are distinct.

**Methods:** Pathological material from C9ORF72-ALS patients was obtained from the Sheffield Brain Tissue Bank. Sense and antisense RNA foci were visualized by RNA fluorescence *in-situ* hybridization (FISH). Interaction with proposed foci binding partners and with TDP-43 was examined by immunohistochemistry (IHC). Direct and specific binding to the sense and antisense repeat sequence was examined by UV-crosslinking.

**Results:** C9ORF72-ALS is associated with pathology of motor and non-motor areas. In the cerebellum, a characteristic location for extra-motor pathology, the cellular distribution of sense and antisense RNA foci are relatively distinct. Sense foci are more abundant in the granule neurons ($p < 0.05$) whereas antisense foci are more abundant in the Purkinje cells ($p < 0.05$). In the motor neurons of the ventral horn, which are the primary target for pathology in ALS, both sense and antisense foci were observed but antisense foci were present at a higher frequency ($p < 0.05$). The presence of antisense ($chi^2$, $p < 0.05$) but not sense ($chi^2$, $p = 0.75$) RNA foci was correlated with nuclear loss of TDP-43 in the motor neurons. In all neuronal populations foci were observed primarily in the nucleus but also in the cytoplasm. Observed co-localisation with protein binding partners was not different between sense and antisense foci.

**Discussion and conclusion:** Our data suggests that if sequestration of protein binding partners is important to C9ORF72-ALS disease pathogenesis then sense and antisense RNA foci should be equally toxic. However nuclear loss of TDP-43 in motor neurons, which is known to correlate directly with neurodegeneration, is associated with the presence of antisense but not sense RNA foci. This suggests a key determinant of disease may be the increased frequency of antisense foci in motor neurons. The factors determining antisense transcription of the repeat expansion are currently unknown. Our work suggests that any therapeutic approach to C9ORF72-ALS must reduce antisense RNA foci in motor neurons.

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Association and the Wellcome Trust (PJS). We are grateful to those who donated biosamples.

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## C4 C9ORF72 EXPRESSION IN AMYOTROPHIC LATERAL SCLEROSIS AND FRONTOTEMPORAL DEMENTIA

DE MUYNCK L[1,2], DIEKSTRA F[3], BORRONI B[4], MEDIC J[3], THIJS V[2,5], CAMUZAT A[6,7], VAN DEN BOSCH L[1,2], VAN DEN BERG LH[3], ROBBERECHT W[1,2], LE BER I[6,7], VELDINK JH[3], VAN DAMME P[1,2]

[1]*KU Leuven - University of Leuven, Department of Neurosciences, Experimental Neurology and Leuven Research Institute for Neuroscience and Disease (LIND), Leuven, Belgium,* [2]*VIB, Vesalius Research Center, Laboratory of Neurobiology, Leuven, Belgium,* [3]*Department of Neurology, Rudolf Magnus Institute of Neuroscience, University Medical Center, Utrecht, The Netherlands,* [4]*Centre for Aging Brain and Neurodegenerative Disorders, Neurology Unit, University of Brescia, Brescia, Italy,* [5]*University Hospitals Leuven, Department of Neurology, Leuven, Belgium,* [6]*Inserm, UMR_S975, Centre de Recherche de l'Institut du Cerveau et de la Moelle Epinière, Paris, France,* [7]*UPMC Univ Paris 06, UMR_S975, Paris, France*

*Email address for correspondence: Louis.DeMuynck@vib-kuleuven.be*

*Keywords: C9ORF72, gene expression, frontotemporal dementia (FTD)*

**Background:** The hexanucleotide GGGGCC repeat expansions in the C9ORF72 gene are a common cause of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD) and ALS-FTD. Haploinsufficiency caused by loss of expression from the mutant allele has been proposed as a potential disease mechanism.

**Objective:** To study C9ORF72 expression in brain and blood and to assess any associations with disease parameters.

**Methods:** C9ORF72 isoform-specific gene expression assays and an assay targeting all known C9ORF72 transcripts were developed and validated with absolute mRNA quantification via droplet digital PCR (ddPCR). Quantitative PCR (qPCR) was performed on frontal and occipital cortex from patients with hexanucleotide repeat expansions and control subjects. ddPCR was carried on blood samples from patients with repeat expansions and controls. Blood ddPCR measurements were replicated with qPCR in a large independent incident cohort of blood samples from patients with repeat expansions, sporadic ALS and FTD patients, and unaffected controls. Association of C9ORF72 expression with age at sample collection, age at onset and survival was assessed with Spearman's test of correlation or Cox proportional hazards regression models.

**Results:** In frontal cortex, occipital cortex and blood samples no significant difference in total C9ORF72 expression was observed between patients carrying hexanucleotide expansions and controls. Repeat expansion carriers showed an altered transcript preference with reduced V2 ratios, but elevated V3 ratios. Aging reduced total C9ORF2 expression in blood from healthy individuals, while total C9ORF72 expression tended to increase with age in repeat expansion carriers. In sporadic ALS and FTD patients, an age-independent elevation in C9ORF72 expression was observed. Lower C9ORF72 levels were associated with increased survival in sporadic ALS patients. We did not detect any association of C9ORF72 blood expression with age at disease onset. Interestingly, the C9ORF72 V3 transcript, which is elevated in hexanucleotide expansion carriers and gives rise to RNA foci and dipeptide repeat (DPR) proteins, was inversely correlated with survival, as estimated with Spearman's test of correlation and using Cox proportional hazards regression models correcting for age at disease onset, age at sample collection or age at diagnosis.

**Discussion and conclusion:** Our findings indicate that C9ORF72 repeat expansions do not significantly affect total C9ORF72 transcript levels in the frontal cortex, occipital cortex or blood. The relative abundance of V2 is reduced, whereas the expression of the V3 transcript containing the expanded GGGGCC repeats is enhanced. V3 correlated negatively with survival. Our data support RNA toxicity and DPR protein toxicity as potential disease mechanisms. Our findings indicate that C9ORF72 expression is an age-independent blood marker of sporadic ALS and FTD, and higher C9ORF72 levels are associated with a survival disadvantage in sporadic ALS patients. Given the readily accessibility of blood samples, such transcript measurements may become a useful biomarker in C9ORF72 repeat expansion carriers.

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## C5 HEXANUCLEOTIDE REPEAT EXPANSIONS CAUSE ABERRANT INTRON 1 RETENTION IN C9ORF72 TRANSCRIPTS: AN EARLY EVENT IN THE PATHOGENESIS OF C9ALS/FTD

NIBLOCK M[1], LEBLOND-MANRY C[2], DION P[2], NISHIMURA A[1], SHUM C[1], SARDONE V[1], LEE Y[1], GOMEZ-DEZA J[1], ROULEAU GA[2], SHAW CE[1], GALLO J-M[1]

[1]*King's College London, London, UK,* [2]*McGill University, Montreal, Canada*

*Email address for correspondence: jean-marc.gallo@kcl.ac.uk*

*Keywords: C9ORF72, RNA, splicing*

**Background:** The most common cause of familial and sporadic Motor Neurone Disease/Amyotrophic Lateral Sclerosis is a $G_4C_2$ hexanucleotide repeat expansion mutation in intron 1 of the *C9ORF72* gene. Intron 1 should normally be spliced out and degraded in the nucleus; however, the RNA transcribed from the hexanucleotide expansion forms nuclear foci recruiting RNA-binding proteins and is exported to the cytoplasm where it is translated into poly-dipeptides by repeat-associated non-AUG-dependent (RAN) translation. Therefore the processing of expanded $G_4C_2$ repeats-containing C9ORF72 pre-mRNA is clearly defective.

**Objectives:** To determine at what stage the processing of C9ORF72 pre-mRNA is affected by the hexanucleotide repeat expansion.

**Methods:** We analysed C9ORF72 transcripts in cultured lymphoblasts and neural- differentiated induced pluripotent stem cells (iPSC) established from heterozygous hexanucleotide repeat expansion carriers and control individuals. Reverse transcribed poly $(A)^+$ RNA was analysed by PCR using sets of primers annealing to intronic or exonic sequences of C9ORF72.

**Results:** PCR of reverse-transcribed RNA from lymphoblasts with primers annealing with exon 1a or exon 5, and with intron 1, upstream or downstream of the repeat domain, generated products indicative of a transcript retaining intron 1. Sequencing of the products demonstrated exact exon 1a-intron 1, intron 1-exon 2 and exon 2–3, 3–4 and 4–5 junctions. Importantly, lymphoblasts from hexanucleotide repeat expansion carriers showed a significant increase in the proportion of transcripts retaining intron 1 compared to control cells. Intron 1-retaining C9ORF72 transcripts were also detected in neural differentiated iPSCs derived from repeat expansion carriers. Nuclear levels of intron 1-retaining transcript relative to cytoplasmic levels were significantly higher than for the normally spliced transcript in lymphoblasts from C9ORF72 repeat expansion carriers.

**Discussion and conclusion:** We have identified a disease-specific RNA species, referred to as C9Int1$^{+}$, corresponding to a mature C9ORF72 mRNA retaining full-length intron 1 in the 5'-UTR. The GGGGCC repeat region of C9Int1$^{+}$ retained in the nucleus would be protected from degradation and accumulate in foci. A proportion of C9Int1$^{+}$ would be exported to the cytoplasm by a conventional pathway of mRNA export and become template for RAN-translation. Inhibiting intron 1 retention would represent a therapeutic strategy for a significant proportion of Motor Neurone Disease cases.

**Acknowledgements:** This work was supported by the Wellcome Trust and the Medical Research Council.

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# SESSION 2B DIAGNOSIS/PROGNOSIS

## C6 THE CHALLENGE OF EARLY THERAPEUTIC INTERVENTION IN ALS

BENATAR M, WUU J

*University of Miami, Miami, USA*

*Email address for correspondence: mbenatar@med.miami.edu*

*Keywords: diagnostic delay, pre-symptomatic, biomarkers*

**Introduction:** The ALS disease process begins with some early pathological event (ie, disease onset), followed by clinical manifestations (ie, symptoms and signs on physical examination), and ends with death. Unfortunately, due to the delay (on average ~10 months) in diagnosing ALS, treatment is often initiated relatively late in the disease course. Furthermore, by the time symptoms emerge, the underlying neurodegenerative process has likely been underway for some time. Reducing the diagnostic delay and improving our understanding of the pre-symptomatic period therefore represent two critical challenges to, but also opportunities for, early therapeutic intervention.

**Symptomatic Disease:** Early treatment of symptomatic disease requires early diagnosis. There is however insufficient awareness among the lay public, primary care physicians and other non-ALS specialists that progressive painless weakness may represent ALS. This leads to delays in patients seeking medical attention and in physicians referring patients to ALS specialists for evaluation. Moreover, some physicians' reluctance to communicate a diagnosis of ALS to patients further contributes to the diagnostic delay. On the other hand, although the diagnosis of ALS is relatively straightforward when the time from symptom onset to specialist evaluation is prolonged, clinical diagnosis is likely to be more challenging earlier in the course of disease; it is in this context that diagnostic biomarkers are most relevant.

**Pre-Symptomatic Disease:** By definition, pre-symptomatic disease is characterized by an absence of symptoms (and a paucity of physical signs of disease). Pre-symptomatic detection of disease, therefore, must rely upon biological markers of the underlying disease process. Currently, no such biomarkers exist, but neuroimaging, neurophysiological testing, and biochemical analysis of biological fluids hold great promise. Importantly, the low incidence of ALS renders impractical the use of pre-symptomatic biomarkers to screen the general population. Rather, these biomarkers are most suitable for use in the subset of individuals known to be at particularly high risk for developing ALS, which based on current knowledge, is limited to those who carry a mutation in an ALS susceptibility gene. The true utility of these biomarkers will lie in their sensitivity to quantifying the pre-symptomatic burden of disease and in identifying the sub-population most likely to benefit from pre-symptomatic therapeutic intervention.

**A Call to Action:** Campaigns to raise awareness of ALS among the lay community and to educate the general medical community about symptoms that should arouse suspicion for ALS are critical to reducing the lag time between symptom onset and referral to an ALS specialist for evaluation. There is also an urgent need to develop biomarkers, both for aiding the diagnosis of ALS in patients who are seen earlier in the course of disease, and for studying pre-symptomatic disease. These combined efforts offer the best hope for early symptomatic treatment and even disease prevention.

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## C7 WHAT DOES THE STUDY OF PREMANIFEST DISEASE CONTRIBUTE? LESSONS FROM OTHER NEURODEGENERATIVE DISEASES

TABRIZI S

*UCL Institute of Neurology, London, UK*

*Email address for correspondence: s.tabrizi@prion.ucl.ac.uk*

*Keywords: Huntington's disease, premanifest, biomarkers*

Huntington's disease (HD) is a devastating autosomal dominantly inherited neurodegenerative disease for which there is currently no effective disease modifying therapy. The genetic predictability of HD provides an opportunity for early therapeutic intervention many years before overt symptom onset and at a time when reversal or prevention of neural dysfunction may still be possible. As HD is monogenetic, fully penetrant, and characterised by a long premanifest phase, it is emerging as a potential model for studying therapeutic intervention in other neurodegenerative conditions such as Alzheimer's or Parkinson's disease where no preclinical diagnostic tests exist.

Since 2008 TRACK-HD has chronicled the earliest stages of the neurodegenerative disease processes in premanifest and mild to moderately symptomatic individuals who carry the HD expansion mutation. TRACK-HD was designed to observe natural disease progression in premanifest and early stage HD with the aim of understanding the preclinical and early phases of neurodegeneration, phenotypic correlates of neuronal dysfunction and to establish sensitive and specific clinical and biological markers of disease progression. Published TRACK-HD data includes longitudinal effect sizes for disease-progression in early stage HD over 24 months and detailed phenotypic dissection of disease progression in both premanifest and early-HD over 36-months, identifying predictors of clinical decline that are independent of age and CAG effects. Both have important implications for clinical trial design, and further our understanding of disease progression across the spectrum of HD. We are now in a position to model progression in a range of functional and imaging measures across the spectrum of HD, and our ongoing research aims to identify neural compensatory networks that may occur in the premanifest phase of neurodegeneration in HD.

Understanding of HD pathogenesis is evolving, and there is a number of candidate therapeutics with potential disease-modifying effects that are currently being tested. The most promising approaches will be briefly reviewed. I will also present new unpublished data from TRACK-HD, mapping basal ganglia connectivity and degeneration of cortico-striatal connectivity with disease progression. I will also present new data from the Track-On study in which the aim was to dissect the relationship between brain structure, function and behaviour and identify whether normal performance in those with higher disease load indicates compensatory brain activity in premanifest stages of the disease. To this end the interactive effect of structural degeneration on cognitive behaviour, sensorimotor networks, fMRI activity and resting state connectivity have been explored and its relevance to the natural history of premanifest HD will be presented.

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## C8 CORTICAL EXCITABILITY IN FAMILIAL C9ORF72 ALS PATIENTS

GEEVASINGA N, MENON P, NICHOLSON G, NG K, KIERNAN MC, VUCIC S

*University of Sydney, Sydney, NSW, Australia*

*Email address for correspondence: nimesh84@hotmail.com*

*Keywords: C9ORF72, transcranial magnetic stimulation, cortical hyperexcitability*

**Background:** In familial amyotrophic lateral sclerosis (FALS) an underlying gene mutation has now been identified in ~60% of cases. The recently identified C9ORF72 gene expansion is recognized as the most common cause of ALS. The mechanisms by which hexanucleotide gene expansions in the C9ORF72 gene lead to neurodegeneration is complex and remains to be fully elucidated, glutamate-mediated excitotoxicity may be a contributing mechanism.

**Objective:** Cortical hyperexcitability, as reflected by the threshold tracking transcranial magnetic stimulation (TTTMS) technique, was an early feature in SOD1 mutation FALS patients. Consequently, the present study explored cortical dysfunction in affected carriers and non-affected carriers with the C9ORF72 repeat expansion.

**Methods:** Cortical excitability studies were undertaken on two cohorts with the C9ORF72 repeat expansion. A symptomatic cohort, who manifested disease (6 males and 4 females; age range 41–78; mean age 62), and an asymptomatic mutation carrier cohort (9 females and 1 male; age range 26–78; mean age 49). Patients were compared with 37 age-matched controls and 82 sporadic ALS patients.

**Results:** Short-interval intracortical inhibition was significantly reduced in C9ORF72 FALS and sporadic ALS patients (SALS), (FALS -0.6 ± 1.6%; SALS 1.6 ± 1.2%; $P < 0.0001$), as was the cortical silent period duration (FALS 182 ± 12 ms, $P < 0.02$; SALS 174 ± 5ms, $P < 0.01$). Central motor conduction time was prolonged (FALS 6.1 ± 0.6 ms, $P < 0.05$; SALS 6.6 ± 0.2 ms, $P < 0.0001$; controls 5.5 ± 0.3 ms) and motor evoked potential amplitude was increased in both ALS groups (FALS 45.3 ± 7.0%, $P < 0.05$; SALS 31.1 ± 2.8%, $P < 0.05$; controls 23.8 ± 2.4%). Resting motor threshold (RMT) was significantly reduced amongst FALS patients ($P < 0.01$) but not in SALS patients, whilst a reduction in the RMT was also seen in asymptomatic carriers ($P < 0.01$). There were no significant differences in cortical excitability in asymptomatic mutation carriers when compared to controls.

**Discussion and conclusion:** Cortical hyperexcitability appears to be a feature of the pathophysiological process in patients with the C9ORF72 gene expansion, potentially contributing to C9ORF72 FALS pathophysiology. Asymptomatic carriers do not exhibit cortical hyperexcitability. Hence additional factors must be involved during the course of an asymptomatic carrier's life, which triggers the process of hyperexcitability.

FALS patients with the C9ORF72 gene expansion and SALS patients share a common pathophysiological process of cortical hyperexcitability. The same features are seen in patients with the SOD1 mutation. Whether cortical hyperexcitability is part of a common final pathway or part of the initiating pathophysiological process will need to be elucidated. Doing so, may aid in the tailoring of effective therapeutic options in the future.

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## C9 EVALUATION OF ROUTINE LABORATORY TESTS AS POSSIBLE BIOMARKERS OF ALS IN THE PRECLINICAL AND CLINICAL PHASE

NEFUSSY B[1], SILVERMAN B[2], ARTMAN I[1,2], VAINER B[1], BLUMEN S[3,4], DRORY V[1,5]

[1]*Tel-Aviv Medical Center, Tel-Aviv, Israel,* [2]*Maccabi Health Care Services, Tel-Aviv, Israel,* [3]*Hillel Yaffe Medical Center, Hadera, Israel,* [4]*Rappaport Faculty of Medicine, Technion, Haifa, Israel,* [5]*Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel*

*Email address for correspondence: vdrory@post.tau.ac.il*

*Keywords: preclinical, creatinine, glucose*

**Background:** Despite extensive efforts, there is no clinically useful biomarker for predicting onset of disease, diagnosis or progression in ALS.

**Objective:** We examined the association between serum levels of routine laboratory tests and the onset of ALS, rate of disease progression and survival. The laboratory parameters tested were uric acid (UA), creatinine (Cr), creatinine phosphokinase (CK), and glucose and glycosylated haemoglobin (HbA1c).

**Methods:** This retrospective study was performed in collaboration with Maccabi health care services. Maccabi is the second largest health care provider in Israel, and has used a central database of laboratory test results since 1998.

The patient group analysed consisted of 104 ALS patients followed up at Tel-Aviv Medical Center that were insured by Maccabi. Age and disease form at onset, gender, rate of progression as measured by ALSFRS-R change over time and survival were known for all patients. The control group consisted of 312 individuals from the Maccabi database without a history of ALS, matched by age, gender and geographical area of residence (3 controls for each patient).

For cases and controls, Maccabi collected from the laboratory database all values of UA, Cr, CK, glucose and HbA1c for the period from 1998–2010. Laboratory values measured 6 and 12 months prior to disease onset ('pre-onset'), at disease onset and 6 and 12 months thereafter ('post-onset') ± 2 months were used for statistical analysis. For controls, the age at disease onset of the matched patient was considered the cut off point for defining 'pre- and post-onset'. Levels of each laboratory parameter were compared between patients and controls, and pre- and post-onset in the same individual.

**Results:** Pre-onset levels of all parameters did not differ between the groups. However, Cr was significantly lower at six months (0.91 ± 0.03 vs 1.013 ± 0.02; $p = 0.019$) and one year after disease onset (0.84 ± 0.03 vs 1.03 ± 0.02; $p < 0.0001$). Glucose was significantly decreased compared to controls one year after disease onset (96.3 ± 30 vs 104.7 ± 2.2; $p = 0.025$). UA decreased with time in patients ($p = 0.005$), but was not significantly lower compared to controls. CK increased with time in patients, but not in controls. A significant difference in CK levels between patients and controls was observed at disease onset (208.3 ± 26.4 vs 88.5 ± 25.8; $p = 0.0017$) and one year after onset (205.9 ± 24.7 vs 104.9 ± 29.7; $p = 0.01$). Survival was negatively correlated with glucose levels at onset ($p = 0.002$; hazard ratio 1.048).

**Discussion and conclusion:** None of the parameters examined was predictive for disease onset. Decrease in serum Cr and increase in CK may be useful as biomarker for disease progression. Glucose levels in serum at disease onset were a strong prognostic factor for survival.

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# SESSION 3A PROTEIN MISFOLDING AND TOXICITY

## C10 THE DYNAMICS OF PROTEIN FOLDING: PATHOLOGIC AGGREGATION IN ALS MICE FOLLOWS TEST-TUBE BEHAVIOUR

OLIVEBERG M

*Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden*

*Email address for correspondence: mikael.oliveberg@dbb.su.se*

*Keywords: transgenic mouse model, SOD1 aggregation, in-vitro fibrillation*

The structure and kinetics of protein aggregation *in vivo* during neurodegenerative is, as of yet, poorly explored and quantified. In this talk, data will be presented showing that disease progression and protein aggregation in transgenic ALS mice expressing a series of human SOD1 mutants, mimics, with remarkable accuracy the behaviour of SOD1 aggregation *in vitro*.

Moreover, we see that the structure of the *in vivo* SOD1 aggregates in some cases, form co-existing strains with different mechanical properties coupled to different disease kinetics. Taken together this indicates that, despite the complexity of the living tissue, *in vivo* protein aggregation obeys simplistic physical-chemical rules, predictable from the molecular properties of the causative proteins as characterised *in vitro*.

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## C11 MISFOLDED WILD-TYPE SOD1 INDUCED BY PATHOLOGICAL FUS OR TDP-43 TRANSMITS INTERCELLULARLY AND IS PROPAGATED MISFOLDING-COMPETENT

POKRISHEVSKY E, GRAD L, CASHMAN N

*The University of British Columbia, Vancouver, BC, Canada*

*Email address for correspondence: edikpok@gmail.com*

*Keywords: protein misfolding, SOD1, TDP-43*

**Background:** Clinically indistinguishable cases of ALS can be caused by inheritable mutation in the genes encoding SOD1, TDP-43, FUS, as well as others, or can occur sporadically. Misfolded SOD1 has been detected in both familial and sporadic ALS patients (1–3), despite mutations in SOD1 accounting for only ~2% of cases. We previously reported that pathological FUS or TDP-43 induces misfolding of human wtSOD1 in living cells (1).

**Objectives:** To examine whether misfolded wtSOD1 (misSOD1) associated with pathological FUS or TDP-43 can spread intercellularly and actively induce the conversion of endogenous SOD1, and if such propagation can be blocked using misfolded SOD1-specific antibodies. We will also determine if TDP-43 pathology spreads between cells, acting as a secondary mechanism for the spread of SOD1 misfolding.

**Methods:** Human cell cultures and mouse primary neural cultures expressing human wtSOD1 were used. We utilized novel confirmation-specific antibodies that detect pathological misSOD1 (1, 4). Intercellular spread and active conversion of SOD1 was determined by incubating untransfected cells with conditioned media from mutant FUS/TDP-43 transfected cells, followed by immunofluorescence microscopy and immunoprecipitation analysis. Blocking of misSOD1 transmission was performed by pre-incubating conditioned media with misfolded SOD1-specific antibodies (5). Pathological TDP-43 was determined by the detection of its hyperphosphorylation, mislocalization and C-terminal cleavage.

**Results:** Mutant FUS or TDP-43-induced misSOD1 can spread intercellularly through conditioned media, triggering misfolding of endogenous wtSOD1 in untransfected cells. Recipient cells that were pre-treated with SOD1-siRNA do not contain misSOD1, implying that endogenous SOD1 is required as substrate for active conversion. Specific immunodepletion of misSOD1 from conditioned media prevents the spread of SOD1 misfolding. Transfection of TDP-43 into cells triggers its cleavage, mislocalization and hyperphosphorylation; these properties are not observed in untransfected cells incubated with conditioned media from TDP-43 transfected cells.

**Discussion and conclusion:** We report that FUS or TDP-43-induced misSOD1 can traverse between cells through incubation of neural cell cultures with conditioned media, triggering active conversion of the endogenous wtSOD1. This spread can be arrested through incubation of the conditioned media with SOD1 misfolding-specific antibodies, demonstrating the therapeutic potential of these antibodies. The absence of TDP-43 pathology in recipient cells, with the presence of misSOD1, further confirms that the transmission of SOD1 misfolding occurs independently of TDP-43.

**Acknowledgements:** This work was supported by the Canadian Institutes for Health Research and ALS Canada. Antibodies were provided by Amorfix Life Sciences Ltd.

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## C12 CO-EXPRESSION OF STRAIN A- AND B-AGGREGATE FORMING HUMAN SOD1 MUTANTS IN MICE: STUDIES OF AGGREGATE STRUCTURE AND DISEASE PHENOTYPE

TOKUDA E[1], ZETTERSTRÖM P[1], ANDERSEN P[2], BRÄNNSTRÖM T[1], MARKLUND S[1]

[1]*Department of Medical Biosciences,* [2]*Department of Clinical Neuroscience, Umeå University, Umeå, Sweden*

*Email address for correspondence: eiichi.tokuda@gmail.com*

*Keywords: SOD1, aggregates, double transgenic mice*

**Background:** It is increasingly recognized that pathogenic proteins in many neurodegenerative diseases, especially prion diseases, assemble conformationally distinct aggregates. When two prion strains with distinct conformations are co-expressed

in the same host, one strain inhibits the ability of the other to cause disease. We have recently found that human superoxide dismutase 1 (hSOD1) variants expressed in mice misassemble into aggregates with distinct structures. Strain A aggregates are formed in $hSOD1^{G93A}$, $hSOD1^{G85R}$, and wild-type hSOD1 mice, and strain B are in $hSOD1^{D90A}$ mice. Of note is that $hSOD1^{D90A}$ is associated with bladder disturbances both in humans and in transgenic mice expressing the mutation.

**Objectives:** The aim of the present study was to study hSOD1 aggregation and disease phenotype in mice co-expressing hSOD1 mutants with preponderance to form conformationally different aggregate strains.

**Methods**: Hemizygous $hSOD1^{G85R}$ mice were crossed with hemizygous $hSOD1^{D90A}$ mice. Disease onset was defined as the time when mice reached peak body weight. The end-point was defined as the age at which a mouse was unable to right itself within 5 s after being pushed onto its side. Disease progression was determined as the period between the disease onset and the end-point.

Due to similar molecular mass and electrophoretic mobility between $hSOD1^{G85R}$ and $hSOD1^{D90A}$, it is difficult to separate both mutants using a conventional antibody against hSOD1. To distinguish both mutants in double transgenic system, we developed mutually exclusive antibodies: one directed against $hSOD1^{D90A}$ which does not recognize $hSOD1^{G85R}$, the other raised against wild-type hSOD1 that recognizes $hSOD1^{G85R}$, but not $hSOD1^{D90A}$.

Spinal cords were harvested at the presymptomatic, symptomatic, and terminal stages. For analysis of SOD1 aggregates, detergent-insoluble fractions were extracted from spinal cords, and the fractions were investigated using immunoblots and dot-blots.

**Results:** Disease onset in double transgenic mice occurred 23% earlier than in $SOD1^{G85R}$ mice ($333 \pm 21$ vs $255 \pm 20$ days). The lifespan was shortened from $381 \pm 26$ to $339 \pm 21$ days, representing a decrease of 11%. The disease progression was thus slowed by 75% ($48 \pm 6.5$ vs $84 \pm 21$ days).

Despite the accelerated disease onset in double transgenic mice, no $hSOD1^{G85R}$ and $hSOD1^{D90A}$ aggregates were evident in a presymptomatic stage. Concomitant with the slower disease progression, both mutants synergistically promoted the aggregation of each other. The aggregates formed were of strain A-type. Still, bladder disturbances were found in all double transgenic mice, which thus are related to $hSOD1^{D90A}$ *per se* and not strain B aggregation.

**Discussion and conclusion:** The disease progression can be modulated even by combining two hSOD1 mutants with distinct conformations. This may be associated with hSOD1 aggregates.

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## C13 GLUTAMATE STIMULATES MOTOR NEURONS TO FORM INTRACELLULAR P-TDP-43 AGGREGATES

SHIBATA N, NIIDA-KAWAGUCHI M, INOSE Y, MURAMATSU F, KATO Y, YAMAMOTO T, SAWADA T

*Department of Pathology, Tokyo Women's Medical University, Tokyo, Japan*

*Email address for correspondence: shibatan@research.twmu.ac.jp*

*Keywords: glutamate, NMDAR, TDP-43*

**Background:** To date, consensus that the appearance of intracellular aggregates of phosphorylated transactive response DNA-binding protein 43 kDa (p-TDP-43) in motor neurons is the characteristic hallmark of sporadic amyotrophic lateral sclerosis (ALS) has been provided (1). On the other hand, previous studies have suggested the involvement of glutamate neurotoxicity in ALS (2). However, the relationship between p-TDP-43 aggregation and glutamate neurotoxicity remains to be clarified.

**Objectives:** To determine whether glutamate stimulation may be upstream of p-TDP-43 aggregation.

**Methods:** The murine-derived motor neuron (NSC34) cell line, characterized by expression of the motor neuron markers N-methyl-D-aspartate receptor (NMDAR) and choline acetyltransferase (CAT), was used for our *in vitro* study. NSC34 cells were maintained in chamber slides using Dulbecco's modified Eagle's medium with high glucose plus 10% fetal bovine serum with a commercial antibiotic cocktail. After serum starvation for 24h to induce expression of NMDAR and CAT, cells were incubated for 24h with or without 100 μM monosodium glutamate (MSG), in the presence or absence of mitogen-activated protein kinase (MAPK) extracellular signal-regulated kinase (MEK) inhibitor. Slides were postfixed in 4% paraformaldehyde, rinsed in phosphate-buffered saline, and incubated overnight at 4°C with a mouse monoclonal IgG against p-TDP-43 followed by Cy3-labeled anti-mouse IgG, mounted with a DAPI-containing immersion, and observed using confocal laser microscopy (LSM-710, Zeiss). The pixel number of p-TDP-43 aggregates and the DAPI-identified cell nucleus number were counted in four dishes per group using image software (Winroof; ImageJ). The data, defined as pixels per cells, were compared among the different groups using one way ANOVA followed by post hoc Bonferroni correction. Statistical significance was considered as $p < 0.05$.

**Results:** The p-TDP-43 aggregates in both the nucleus and cytoplasm were significantly increased in the MSG only group ($P < 0.001$ by Bonferroni) as compared to the vehicle and MEK inhibitor only groups. MSG-driven increases in the aggregates were significantly abrogated by pre incubation with MEK inhibitor ($P < 0.005$ by ANOVA).

**Discussion and conclusion:** A recent study indicated that inhibition of astrocytic glutamate transporter triggered intracellular aggregation of p-TDP-43 (3), suggesting the involvement of glutamate stimulation in p-TDP-43 aggregation. This is consistent with our findings. The present results provide *in vitro* evidence that glutamate stimulates motor neurons to form intracellular p-TDP-43 aggregates via the MEK pathway.

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# SESSION 3B TRIALS AND TRIAL DESIGN

## C14 DOES A PLACEBO CONTROLLED CLINICAL TRIAL CORRECTLY ESTIMATE THE TREATMENT EFFECT IF THE DELIVERY METHOD FOR THE TREATMENT AND THE PLACEBO IS POTENTIALLY HARMFUL?

SCHOENFELD D, HAYDEN D, SHUI A, CUDKOWICZ M

*Massachusetts General Hospital, Boston, Massachusetts, USA*

*Email address for correspondence: dschoenfeld@partners.org*

*Keywords: clinical trials, treatment, biostatistics*

**Background:** Many new ALS treatments require difficult delivery methods such as continuous infusion by a central venous catheter, infusion into the cerebrospinal fluid, or other surgery. If these delivery methods are potentially harmful then the neuroprotective effect of a treatment might be negated by the harmful effect of its delivery and the difference between the active treatment group and a placebo group, receiving the delivery only, might exaggerate the benefit of treatment when compared to the standard of care.

**Objectives:** To estimate whether central venous catheter usage in the recent trial of Ceftriaxone was harmful and to determine if this would negate any differences between the placebo and treatment group.

**Methods:** The incidence of catheter related adverse events was captured in the case report form. A proportional hazards model was used to calculate the effect of the occurrence of these events on subsequent survival. A simulation was performed to impute the hazard ratio of active treatment against no treatment where the effect of catheter related serious adverse events were removed.

**Results:** There were 44 serious adverse events reported to be related to the catheter. The risk of a serious event was 5.6% patients per year. Twenty events were infections these were more prevalent in the placebo arm of the trial ($p < 0.002$). There was a suggestion that catheter related serious events increased mortality ($p = 0.057$, HR = 1.56), whilst non-serious events had no effect on mortality ($p = 0.82$). In a simulation based on these results, the observed placebo versus active hazard ratio of 1.11 would yield a placebo versus standard of care hazard ratio of 1.08. In a study where the rate of serious delivery related adverse events was much higher, say one per patient per year, a hazard ratio of 1.29 between placebo and treatment would yield no benefit when comparing no treatment to treatment.

**Discussion and conclusion:** Serious catheter related adverse events were rare in the Ceftriaxone trial and it is unlikely that the effect of the catheter in this study distorted the estimate of the hazard ratio. However, in future studies the harmful effects of the delivery method may be greater and difficult to assess. For instance there is a report that the insertion of a feeding tube seemed to worsen survival (1), which may have been the result of the harmful effects of its placement. Therefore we may need to consider using a standard of care control arm, rather than a placebo control or conducting a three-arm study with a standard of care arm when the treatment delivery method is potentially harmful.

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## C15 INTRACEREBROVENTRICULAR DELIVERY OF VEGF IS FEASIBLE AND SAFE IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS, A PHASE I STUDY

VAN DAMME P[1, 2], TILKIN P[1], JANSSON K[4], TERRYN J[1], HERNE N[4], TOUSSEYN T[1], ZACHRISSON O[4], ALMQVIST P[4], SHAFER L[3], COFFEY R[3], NUTTIN B[1], JERLING M[4], BERNADOTTE F[4], HAEGERSTR A[4], ROBBERECHT W[1]

[1]*KU Leuven, Leuven, Belgium,* [2]*VIB Leuven, Leuven, Belgium,* [3]*Medtronic Inc, Minneapolis, MN, USA,* [4]*Newron Sweden AB, Stockholm, Sweden*

*Email address for correspondence: philip.vandamme@uz.kuleuven.be*

*Keywords: VEGF, intracerebroventricular, clinical trials*

**Background:** Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder primarily affecting the motor system. Progressive weakness and wasting of limb, respiratory and bulbar muscles limit survival to, on average 36 months after disease onset. The growth factor vascular endothelial growth factor (VEGF) has been implicated in the pathogenesis of motor neuron degeneration and intracerebroventricular delivery (ICV) of VEGF has been shown to slow down the progression of motor neuron degeneration in rodent models.

**Objectives:** We studied the feasibility, safety, tolerability, pharmacokinetics and efficacy parameters of intracerebroventricular delivery of recombinant human VEGF165.

**Methods:** In this phase I, first-in-human study in patients with ALS, VEGF165 was delivered using a fully implantable programmable pump connected to a catheter inserted in the frontal horn of lateral cerebral ventricle. Increasing doses of intracerebroventricular VEGF (0.2, 0.8, and 2 μg/day) were administered to a first cohort of 8 patients, followed by a randomized placebo-controlled study in a second cohort of 10 patients. After the 3 month study period, all patients received VEGF in an open label extension study. The 3 month study and the open label extension study were registered with Clinicaltrials.gov identifiers NCT00800501 and NCT01384162, respectively.

**Results:** Fifteen out of eighteen patients completed the 3 month study period. The surgical procedure was well tolerated in all patients and no technical problems with catheter positions or drug delivery arose during the study. At a maximal tested dose of 2 μg/day, administration of ICV VEGF resulted in sustained detectable VEGF levels in the lumbar cerebrospinal fluid. There were no unresolvable side effects or safety issues. The average decline in ALS FRS-R over the 3 month study period was 0.82, 0.88 and 0.49 for the placebo, 0.2 and 0.8 μg/day combined and the 2 μg/day group, respectively ($p = 0.74$). VEGF was also well tolerated for up to 3 years in patients in the open label extension study.

**Discussion and conclusion:** Our data demonstrate that long term ICV VEGF is well tolerated and safe in ALS patients and that studies to further explore safety and efficacy are justified.

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## C16 THE EFFECTS OF TIRASEMTIV ON MEASURES OF RESPIRATORY FUNCTION IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

ANDREWS J[1], LEE J[1], MENG L[1], BIAN A[1], WOLFF A[1], SHEFNER J[2] ON BEHALF OF THE BENEFIT-ALS STUDY GROUP

[1]*Cytokinetics, Inc., South San Francisco, CA, USA,* [2]*SUNY Upstate Medical Center, Syracuse, NY, USA*

*Email address for correspondence: jandrews@cytokinetics.com*

*Keywords: respiratory function, skeletal muscle activator, slow vital capacity*

**Background:** BENEFIT-ALS evaluated the effects of *Tirasemtiv*, a fast skeletal troponin activator, in patients with ALS randomized either to placebo or to an escalating dose of *Tirasemtiv* up to 500 mg per day.

**Objectives:** In this report, we evaluate the effects of *Tirasemtiv* on several measures of respiratory function, and correlations between them and subgroup analyses.

**Methods:** 711 patients were enrolled and began 1 week of open-label *Tirasemtiv* 125 mg BID prior to randomization; 106 patients discontinued from open-label and 156 patients were removed from the analysis due to a drug dispensing error that occurred mid-study. Patients with ≥ 1 efficacy assessment were included in this report. Slow Vital Capacity (SVC), Maximum Voluntary Ventilation (MVV), and Sniff Nasal Inspiratory Pressure (SNIP) were assessed at baseline, after 4, 8 and 12 weeks of double-blind treatment, and 1 and 4 weeks after discontinuing treatment. Changes from baseline were stratified by riluzole use/non-use.

**Results:** During 12 weeks of double-blind treatment, SVC declined more slowly on *Tirasemtiv* versus placebo ($p = 0.0006$). There was no difference in the rate of decline in MVV ($p = 0.880$) or SNIP ($p = 0.211$). Pulmonary measures were reasonably correlated at baseline (SVC/SNIP $r = 0.338$; SVC/MVV $r = 0.321$; MVV/SNIP $r = 0.426$ ($p < 0.0001$ for all)) but changes from baseline generally were poorly correlated. *Tirasemtiv* reduced the decline in SVC versus placebo regardless of age, gender, riluzole use, or BMI. Subgroups with the largest and most significant differences in SVC on *Tirasemtiv* versus placebo (change from baseline to mean SVC after 8 and 12 weeks of double-blind treatment) were: female (6.84%, $p = 0.012$); non-riluzole users (6.55%, $p = 0.0005$); with a baseline SVC ≥ median at baseline (6.02%, $p < 0.0001$). SVC, SNIP and MVV were not affected by weight change.

**Discussion and conclusion:** Treatment with *Tirasemtiv* significantly reduced decline of SVC after 12 weeks of treatment. Subgroup analyses revealed that effect of *Tirasemtiv* on SVC was not a function of any specific subgroup. Vital capacity is a clinically meaningful measure that is used to determine major clinical decisions in the treatment of ALS and is also used to aid prognosis. The effect of *Tirasemtiv* on SVC should have meaningful and positive effect on patients with ALS, if confirmed in future longer duration studies.

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## C17 INFLAMMATION-ASSOCIATED PLASMA FACTORS ARE ASSOCIATED WITH CLINICAL RESPONSE TO NP001: A POST HOC ANALYSIS OF PHASE II CLINICAL AND LABORATORY DATA

MCGRATH MS[1, 2], ZHANG R[1], BLOCK G[2], MILLER RG[3]

[1]*University of California, San Francisco, San Francisco, CA, USA,* [2]*Neuraltus Pharmaceuticals, Palo Alto, CA, USA,* [3]*California Pacific Medical Center, San Francisco, CA, USA*

*Email address for correspondence: mmcgrath@hemeonc.ucsf.edu*

*Keywords: immune activation, monocyte, neuroinflammation*

**Background:** NP001 is an IV form of sodium chlorite that regulates macrophage activation. A recently completed phase II study where 136 ALS patients received placebo, NP001 1mg/kg, or 2mg/kg on a monthly basis over 6-months, revealed subsets of patients who did not progress (responders) based on ALSFRS-R change over the trial. The non-progressor rates were 10%, 19% and 27% in the placebo, 1mg/kg, and 2mg/kg groups respectively; with the former being consistent with historical matched placebo controls.

**Objective:** To test whether inflammatory markers are associated with clinical outcome, plasma from the placebo and 2mg/kg groups were evaluated at baseline and after 6 months of treatment.

**Methods:** Plasma specimens were obtained during the phase II NP001 trial and stored at −80°C. Nineteen inflammatory markers were measured by AssayGate, a CRO specializing in assessment of immune factors.

**Results:** IL-18 levels were significantly higher at baseline ($p = 0.02$) in 2mg/kg NP001 responders compared to non-responders. After 6 months, IL-18 was decreased in most responders, whereas levels increased in the majority of non-responders and patients who received placebo. Baseline LPS levels, which can induce IL-18, were elevated in 30/32 NP001-treated patients; and decreased in most patients after treatment. In contrast, 19/33 placebo patients were initially LPS negative, however after 6 months, 16 of these 19 were LPS positive. Overall, LPS levels increased in most placebo patients after 6 months ($p = 0.01$). Of interest, all of the NP001 responders had LPS in their plasma, whereas none of the placebo non-progressors had detectable LPS at baseline. Curiously, the patients with undetectable baseline LPS in the placebo group had slower rates of disease progression (-0.66 ALSFRS-R units/month) compared to LPS-positive placebos (-0.9 ALSFRS-R units/month).

**Discussion and conclusion:** NP001 halted disease progression in 27% of patients treated for 6 months, 2.5x the percentage in the placebo group. Two major plasma factors may differentiate NP001 responders from non-responders. The responder population had significantly higher levels of IL-18, a cytokine involved in inflammation driven cell death, than the non-responders. Additionally, all NP001 responders had detectable LPS in their plasma, and LPS levels decreased in most NP001 patients, consistent with normalization of macrophage function and the mechanism of action of NP001. Placebo patients without detectable LPS may represent a different slowly progressing population. Importantly, elevated IL-18 and presence of LPS, markers indicative of an ongoing neuroinflammatory process, may help identify patients likely to benefit from NP001. Additionally, such markers may help identify different subpopulations in this heterogeneous disorder.

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# SESSION 4A AUTOPHAGY

## C18 P62/SQSTM1 DEFICIENCY ACCELERATES MOTOR NEURON DEGENERATION IN SOD1H46R TRANSGENIC MICE

PAN L[1], OTOMO A[1], KOIKE M[2], UCHIYAMA Y[2], AOKI M[3], ABE K[1], ISHII T[4], YANAGAWA T[4], SHANG H-F[5], YOSHII F[6], HADANO S[1]

[1]*Department of Molecular Life Sciences, Tokai University School of Medicine, Isehara, Kanagawa, Japan,* [2]*Department of Cell Biology and Neuroscience, Juntendo University Graduate School of Medicine, Bunkyo-ku, Tokyo, Japan,* [3]*Department of Neurology, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan,* [4]*Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan,* [5]*West China Hospital, Sichuan University, Chengdu, Sichuan, China,* [6]*Department of Neurology, Tokai University School of Medicine, Isehara, Kanagawa, Japan*

*Email address for correspondence: shinji@is.icc.u-tokai.ac.jp*

*Keywords: p62/SQSTM1, SOD1, autophagy*

**Background:** Several studies have revealed missense mutations in *SQSTM1* in sporadic ALS cases. *SQSTM1* encodes p62/SQSTM1 regulates selective-autophagy via association with poly-ubiquitinated misfolded proteins. We have previously demonstrated that loss of ALS2 hinders the autophagy-endolysosomal system and accelerates disease progression in SOD1$^{H46R}$ transgenic mice, with accompanying accumulation of insoluble p62/SQSTM1 in the spinal cord. Furthermore, loss of p62/SQSTM1 exacerbates motor dysfunction in SOD1$^{H46R}$ mice, suggesting a possible neuroprotective role of p62/SQSTM1 *in vivo*. However, molecular mechanisms by which p62/SQSTM1 deficiency leads to the accelerated disease phenotypes in mutant SOD1-expressing mice are still unknown.

**Objectives:** To clarify the histological basis for the phenotypic modification in SOD1$^{H46R}$ mice by loss of p62/SQSTM1, and the interrelationship between ALS2 and p62/SQSTM1 *in vivo*.

**Methods:** We generated SOD1$^{H46R}$ mice on a *Sqstm1*-null background by crossing *Sqstm1*$^{+/-}$SOD1$^{H46R}$ with *Sqstm1*$^{+/-}$ mice. We also generated ALS2/SQSTM1-double deficient SOD1$^{H46R}$ mice. For electron microscopic analysis, mice at 16–20 weeks of age were anesthetized, transcardially perfused, and fixed with 2% paraformaldehyde (PFA)/2% glutaraldehyde (GA). Brain and spinal cord were removed and post-fixed with the same fixative for 12 hr at 4°C and with 2% GA for 2 hr at 4°C. Segments were dissected and post-fixed in 1% osmium tetroxide. After dehydration in graded alcohol, tissues were embedded in epoxy resin. Semi-thin sections were stained with toluidine blue and examined under a light microscope. Selected areas were sectioned for ultrastructural examination using an electron microscope. For immunohistochemistry, mice were fixed with 4% PFA. Brain and spinal cord were removed and post-fixed with the same fixative for 48 hr at 4°C. The spinal segment (L4-L5) was embedded in paraffin, sliced, and subjected to histological and immunohistochemical examinations.

**Results:** *Sqstm1*$^{-/-}$SOD1$^{H46R}$ mice showed a much earlier motor dysfunction and a shorter life span than SOD1$^{H46R}$ mice. Axonal degeneration in the spinal tracts, which preceded motor neuronal loss, was evident from an early symptomatic stage in *Sqstm1*$^{-/-}$SOD1$^{H46R}$ mice, but not in SOD1$^{H46R}$ mice of the same ages. Electron microscopic observations revealed the presence of degenerative and/or swollen axons with the accumulation of multi-membrane vesicles as well as damaged organelle in the spinal cord of *Sqstm1*$^{-/-}$SOD1$^{H46R}$ mice at 16 weeks of age. Further, motor neuron degeneration was prominent in *Sqstm1*$^{-/-}$SOD1$^{H46R}$ mice at 20 weeks of age, but not in SOD1$^{H46R}$ mice. Importantly, a simultaneous inactivation of ALS2 and p62/SQSTM1 in SOD1$^{H46R}$ mice further accelerated disease phenotypes compared to either ALS2- or p62/SQSTM1-single deficient counterparts.

**Discussion and conclusion:** These results suggest that dysfunction in the p62/SQSTM1, and/or ALS2-mediated autophagy-endolysosomal system, plays a crucial role in motor neuron degeneration and the pathogenesis of ALS.

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## C19 C9ORF72 INTERACTS WITH FIP200 AND REGULATES THE INITIATION OF AUTOPHAGY

WEBSTER CP, SMITH EF, WALSH MJ, HIGGINBOTTOM A, SHAW PJ, GRIERSON AJ, DE VOS KJ

*Department of Neuroscience, SITRAN, University of Sheffield, Sheffield, UK*

*Email address for correspondence: k.de_vos@sheffield.ac.uk*

*Keywords: C9ORF72, autophagy, protein aggregation*

**Background:** Hexanucleotide repeat expansions in the *C9ORF72* gene account for the majority of familial cases of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). *C9ORF72* codes for two conserved C9ORF72 protein isoforms of unknown function. In addition to TDP-43 pathology, typical of most ALS, C9FTD/ALS patients exhibit p62 positive, TDP-43 negative inclusions in the cerebellum and hippocampus that are not found in other C9FTD/ALS cases. During autophagy p62 targets ubiquitinated proteins and organelles to the autophagosome and is degraded alongside the proteins and organelles it targets. Accordingly p62 positive inclusions are indicative of dysfunctional autophagy. How the repeat expansion in *C9ORF72* leads to disease is not known but may involve loss-of-function of C9ORF72 through *C9ORF72* haploinsufficiency. Thus, C9ORF72 may be involved in autophagy.

**Objectives:** To investigate the possible involvement of C9ORF72 in autophagy.

**Results:** Here we show that reducing cellular levels of C9ORF72 using siRNA prevented the induction of autophagy as measured by LC3-I to LC3-II conversion on immunoblot and by monitoring autophagic flux using an mCherry-EGFP-LC3 autophagy reporter. Conversely, we found that overexpression of C9ORF72 induced autophagy.

Induction of autophagy is mediated by a protein complex that comprises the focal adhesion kinase family-interacting

protein of 200 kDa (FIP200), Unc-51-like kinase 1 (ULK1), and ATG13. To test if C9ORF72 may directly regulate the FIP200/ULK1/ATG13 initiation complex we investigated if C9ORF72 interacts with the complex in co-immunoprecipitation assays. Both FIP200 and ULK1 efficiently co-immunoprecipitated with C9ORF72.

**Discussion and conclusion:** We conclude that C9ORF72 regulates autophagy via interaction with the FIP200/ULK1/ATG13 initiation complex.

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## C20 INCREASING MOTOR-INDEPENDENT AUTOPHAGY ENHANCES DISEASE PROGRESSION IN A MOUSE MODEL OF ALS

PERERA ND, SHEEAN RK, BEART PM, HORNE MK, TURNER BJ

*Florey Institute of Neuroscience and Mental Health, Melbourne, Victoria, Australia*

*Email address for correspondence: nirmaperera_15@yahoo.com*

*Keywords: autophagy, SOD1, mTOR*

**Background:** Autophagy is the main catabolic pathway in neurons that eliminates misfolded proteins, aggregates and damaged organelles linked to neurodegeneration. Misfolded SOD1 accumulates in motor neurons in both sporadic and familial ALS. Therefore, one potential therapeutic approach could be to enhance its degradation through activation of autophagy. Autophagy is regulated by the mTOR pathway. Rapamycin is a widely used inhibitor of mTOR signalling, which induces autophagy. Unexpectedly, rapamycin treatment exacerbates disease in mutant SOD1 mice, possibly due to mTOR inhibition not related to autophagy. Recently, mTOR-independent autophagy pathways regulated by increased intracellular calcium and inositol levels have been identified. Here, we assessed the effects of a novel mTOR-independent autophagy inducer rilmenidine in mutant SOD1 mice.

**Objective:** To investigate the timecourse of autophagy activation and the effects of enhancing mTOR-independent autophagy using rilmenidine in transgenic $SOD1^{G93A}$ mice.

**Methods:** Macroautophagy (p62, LC3), chaperone-mediated autophagy (Hsc70, LAMP2A) and mitophagy (VDAC1) markers were assessed by Western blotting and immunohistochemistry in spinal cords of wild-type and $SOD1^{G93A}$ mice at presymptomatic (30 and 60 days), disease onset (90 days) and advanced (120 days) stages. $SOD1^{G93A}$ mice were administered rilmenidine (10 mg/ml, IP injection, four times per week) from 60 days of age. Mice were examined for weight loss, motor function and survival. Spinal cords and brains were analysed for motor neuron survival, glial cell activation, autophagy induction and mutant SOD1 level and aggregation.

**Results:** p62 and LC3II levels were significantly elevated in spinal cords of $SOD1^{G93A}$ mice from disease onset ($p < 0.05$). Rilmenidine treatment robustly upregulated LC3II levels and reduced VDAC1 levels in spinal cords of mice, indicative of autophagy activation. Soluble mutant SOD1 levels were diminished in spinal cords of rilmenidine treated mice, consistent with autophagy. Despite this, disease onset was not altered by rilmendine, but survival was significantly reduced ($p < 0.05$) and disease progression accelerated in male $SOD1^{G93A}$ mice.

**Discussion and conclusion:** Macroautophagy is the dominant autophagy pathway occurring in motor neurons of mutant SOD1 mice. mTOR-independent autophagy induced by rilmenidine drives disease progression in mutant SOD1 mice, suggesting that autophagy activation contributes to pathology in this ALS model.

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## C21 RAB 1 RESCUES ER STRESS, MACROAUTOPHAGY AND INHIBITION OF ER-GOLGI TRANSPORT INDUCED BY MUTANT FUS IN AMYOTROPHIC LATERAL SCLEROSIS

SOO KY[1], FARG M[1], HALLORAN M[1,2], ATKIN JD[1,2]

[1]*Department of Biochemistry, La Trobe Institute for Molecular Science, La Trobe University, Bundoora, Australia,* [2]*Australian School of Advanced Medicine, Macquarie University, North Ryde, Australia*

*Email address for correspondence: k.soo@latrobe.edu.au*

*Keywords: Rab1, autophagy, endoplasmic reticulum*

**Background:** Autophagy is the major lysosomal pathway by which cells degrade intra-cytoplasmic proteins. When autophagy is induced, omegasomes are derived from endoplasmic reticulum (ER) from which the double layered autophagosome membrane is formed. Autophagosomes ultimately fuse with lysosomes where their contents are degraded. Autophagy is a key route for the degradation of aggregate-prone proteins and has been implicated in multiple pathways linked to ALS. Previously we demonstrated that ALS-associated mutant FUS induces ER stress and inhibits transport of secretory proteins from the ER to the Golgi apparatus. Rab1 plays a key role in both ER-Golgi transport and in the formation of autophagosome membrane.

**Objectives:** The objectives of this study were to determine whether (i) mutant FUS dysregulates autophagy and (ii) overexpression of Rab1 rescues macroautophagy impairment, ER stress and inhibition of ER-Golgi transport in neuronal cells expressing mutant FUS.

**Methods:** Neuro2a cells were transfected with HA-tagged wildtype and mutant FUS (P525L and R522G). Autophagy was examined in FUS transfected cells by (i) the formation of LC3-II vesicles, (ii) the co-localization of ubiquitinated proteins, using NBR1, or early autophagy marker, ATG9, with LC3-II vesicles, using immunofluorescence and confocal microscopy, and (iii) the formation of omegasomes, labelled by co-transfection with DFCP1-Myc construct. ER-Golgi transport was examined using vesicular stomatitis viral glycoprotein (VSVG)-mCherry and ER stress was investigated using immunocytochemistry for CHOP. Rab1-CFP and a dominant negative Rab1 mutant as a control were also co-transfected with FUS proteins. The expression of Rab1 in spinal cord tissues from sporadic ALS patients and controls was also investigated using immunohistochemistry.

**Results:** The levels of LC3-II were reduced in cells expressing mutant FUS compared to controls, demonstrating that mutant FUS inhibits autophagosome formation in ALS. The removal of ubiquitinated proteins by autophagosomes was

also decreased in cells expressing mutant FUS. Furthermore, ATG9 was mis-localised and the formation of the omegasome was reduced in these cells. However, overexpression of Rab1 rescued autophagosome formation, increased omegasome formation, decreased ER stress and rescued ER-Golgi transport inhibition in mutant FUS expressing cells. Immunhistochemistry of sporadic human tissues revealed that Rab1 formed inclusion-like structures in ALS patient motor neurons, but not controls.

**Discussion and conclusion:** These findings demonstrate that mutant FUS impairs macroautophagy in cells via Rab1 inhibition. Rab1, mutant FUS, and ATG9 all regulate formation of the omegasome, which marks autophagosome precursors. This study provides further understanding of the intricate autophagy system and its relationship to dysfunction of the ER and neurodegeneration in ALS. The formation of Rab1 inclusions in ALS patient motor neurons suggests that Rab1 dysfunction is implicated in ALS pathology. Rab1 overexpression rescued the impairment of autophagy, ER stress and inhibition of ER-Golgi transport by mutant FUS, implying that it may be a novel therapeutic target in ALS.

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# SESSION 4B ASSISTIVE TECHNOLOGY

## C22 AAC: FROM LOW TO HIGH TECH

REEVES A

*ACE Centre, Oldham, UK*

*Email address for correspondence: areeves@acecentre.org.uk*

*Keywords: communication, technology, commissioning*

This presentation will provide an overview of the range of assistive technologies and developments in the field of Augmentative and Alternative Communication (AAC) to enable independent communication for individuals with little or no speech. The rapid evolution of mainstream and specialist technologies to support verbal and written communication has proved challenging for successive governments to address in order to harness its life changing potential for children and adults who need it (1). Provision of appropriate equipment for assessment, short term and long term use by people who need technology to communicate and high quality equitable services to support its use has been variable for many years (2). However, the availability and awareness of technology to support independent verbal and written communication for children and adults with a wide range of disabilities has increased, and will continue to do so.

This presentation aims to outline the process in agreeing a vision for the future of national AAC provision from the perspective of its stakeholders and how this vision has been realised. Recent research in the UK (3, 4) has resulted in an additional £15 million recurrent annual funding from the English Government in order to commission specialised AAC services and equipment provision. This is facilitating the development of nationally coordinated specialised and local AAC services in order to provide professionally competent and timely assessments and equipment for children and adults who need AAC. Prioritisation criteria, as stated in the NHS England Complex Disability Equipment communication aid, include referrals of individuals with rapidly degenerating conditions such as MND and national procurement opportunities are consequently being realised, resulting in cost efficiency savings as well as the development of responsive services that are ultimately enhancing the quality of life and outcomes for individuals who may require technology to communicate.

There will be an overview of the range of AAC resources and strategies that are currently available, including paper-based systems, high tech communication aids, access devices and strategies and supportive software. The emergence of new technologies, such as Brain Computer Interface (BCI) and voice banking will be particularly challenging to incorporate into NHS commissioning arrangements in the future as these technologies advance. However, the journey so far has proved that strategic and financial barriers are not insurmountable as society and technology increasingly recognises that the ability to communicate is fundamental in achieving better outcomes and quality of life for anyone challenged by disability.

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## C23 USE OF BRAIN COMPUTER INTERFACES IN ALS

KUBLER A

*Institute of Psychology, University of Würzburg, Würzburg, Germany*

*Email address for correspondence: andrea.kuebler@uni-wuerzburg.de*

*Keywords: brain-computer interaction, communication, quality of life*

Brain-Computer Interfaces (BCI) measure in real time the neuronal activity of the brain, extract and classify relevant neuronal activity and translates it into an output to control an application. No muscular activity is necessary to control a BCI. BCI-controlled applications can restore, improve, replace, and enhance function.

With respect to BCI in ALS the replace function is most important. BCI development faces the challenge of providing a technology ready to be used at the patients' home. The user-centred design approach, which implies an iterative process between developers and end-users of technology, proved valuable in this respect and yielded applications for communication and entertainment. As an example the BCI controlled Brain Painting will be introduced including measures for evaluation of success.

The Brain-Painting application is used at home on a daily basis by two patients with ALS in the locked-in state. Family and caregivers support BCI use and no experts are present. Close monitoring of use, satisfaction, frustration, and joy demonstrated stable control over two years, thus offering a long-term perspective for some patients with ALS. Further, replacement of function has been extended to neuropsychological testing. This opens the possibility of cognitive testing even in late stages of the disease. Within the user-centred design process great care is taken to develop BCIs that can be deployed at the patients' bedside. This includes easy-to-use technology and robust signal recording. Progress in remote control, user-friendly software and electrode design render BCI more feasible in this respect. Hybrid-BCI incorporate as input signals all physiological responses that are controllable by the end-users, such as brain or muscular activity; it can be easily switched between the different signals according to the current capacity of the end-user.

Recent BCI research with ALS patients revealed that if a BCI matches the individual users' needs and requirements, BCI are used in daily life, even if speed and reliability are not yet optimal. Further, it demonstrated that if patients are provided with support that enables them to engage in desired activity and re-integrates them in sociable activities, high quality of life can be experienced even by people in the locked-in state.

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## C24 GIVING VOICE: VOICE BANKING AND VOICE RECONSTRUCTION FOR MND PATIENTS

REWAJ P, COLVILLE S, VEAUX C, BAKOS G, CRANLEY D, YAMAGISHI J, KING S, CHANDRAN S

*University of Edinburgh, Edinburgh, UK*

*Email address for correspondence: phillipa.rewaj@ed.ac.uk*

*Keywords: speech, AAC, Voicebank*

**Background:** The onset of dysarthria in MND can, for many, be one of the most difficult symptoms to accept and manage. Estimates vary on the prevalence of dysarthria in MND, but figures range from 50–80% of patients developing dysarthria at some point in the disease trajectory (1). As speech becomes increasingly unintelligible, many rely on augmentative and alternative communication (AAC) to express themselves. However the use of voice output communication aids (VOCAs) while facilitating communication, cannot currently preserve the identity of the individual, as users are restricted to a limited set of impersonal synthetic voices. Indeed, the inability to accept an alternative voice has been cited as reason for AAC abandonment in MND patients (2).

**Objectives:** This research intends to address these issues through the creation of personalised synthetic voices for use in VOCAs. Using a new a statistical parametric speech synthesis technique the Voicebank Project (3) aims to preserve identity and improve quality of life for AAC users.

**Methods:** Patient voices are captured early in disease progression, preferably before speech deterioration, through recordings made using specially designed software. Participants read aloud between 100 and 400 sentences designed to capture all English phonemes and identify speaker accent. Recordings are 'banked' and parameters unique to the patient's voice are automatically analysed and synthetically reproduced in a process called 'voice cloning'. During the voice cloning process the synthetically reproduced parameters of a patient's voice are combined with those of healthy donor voices. Features of donor voices with the same age, sex and regional accent as the patient are pooled to form an 'average voice model' (AVM), which acts as a base to generate the synthetic voice. When speech is affected by mild to moderate dysarthria at time of recording, it is also possible to 'repair' the voice in the synthesis process using more of the donor AVM to alter affected parameters.

**Results:** Preliminary feedback from 10 patients has been positive. Participants rated similarity of their synthetic voice to original to voice with an average score of 3.5/5, and intelligibility of their synthetic voice with an average score of 4.1/5. All participants expressed a preference for their personalized synthetic voice over a pre-existing generic alternative.

**Discussion and conclusion**: This new speech synthesis technique provides accepted personalised synthetic voices for use in communication aids using minimal speech data and in the presence of dysarthria, helping to preserve identity for AAC users.

**Acknowledgements:** This study was funded by The Euan MacDonald Centre, Anne Rowling Regenerative Neurology Clinic, MRC, and the MND Association (UK).

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# SESSION 5A *IN VITRO* MODELLING

## C25 ARE IPSCS LIVING UP TO THEIR PROMISE?

EGGAN K[1, 2]

[1]*Harvard, Cambridge, Boston, USA,* [2]*HHMI, Cambridge, Boston, USA*

*Email address for correspondence: eggan@mcb.harvard.edu*

*Keywords: stem cells, pluripotency, neurological diseases*

A common feature of conditions negatively impacting the brain, including ALS, is that each has many distinct genetic causes. As a result, the genetic make-up that predisposes each patient to one of these disorders is unique. This heterogeneity raises a simple question: Despite each patient's unique genetic make-up, is there a common cellular or molecular process that is shared among patients with a particular disease? Addressing this question is of critical importance for developing effective therapeutics. Common disease pathways provide targets for broadly applicable therapies. In contrast, if subsets of patients follow distinct paths towards brain dysfunction, then diagnosis and treatment must be specialized to sub-populations, or even individuals. This 'personalized medicine' is becoming standard in treatment of cancer, where tissue is readily available from excised tumors.

In the cases of brain disorders, personalized treatment has been slowed by the inaccessibility of cells in the brain and therefore our inability to study them. Our lab has been a substantial contributor to a general approach for overcoming this roadblock. After obtaining easily collectible blood or skin cells from patients, stem cell and reprogramming methods are used to convert them into the neural cells that malfunction in ALS.

Recently, we have demonstrated the utility of this approach for discovering a new therapeutic candidate for ALS. Using this strategy, we found that the neurons from several classes of ALS patients shared pathological changes in their electrical activity not found in similar neurons produced from healthy subjects. Further studies from our lab, in collaboration with others at the Harvard Stem Cell Institute (HSCI), led to the discovery that an already existing drug for epilepsy could reverse this electrical change. These findings form the basis for an upcoming clinical trial of this drug, in ALS patients, which will begin before the end of this year.

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## C26 A FUNCTIONAL CHARACTERIZATION OF C9ORF72 IPSC-DERIVED MOTOR NEURONS

MUTIHAC R[1], SCABER J[1], LALIC T[2], COWLEY S[3], WADE-MARTINS R[4], TURNER M[1], TALBOT K[1]

[1]*Nuffield Department of Clinical Neurosciences,* [2]*Weatherall Institute for Molecular Medicine,* [3]*Oxford Stem Cell Institute,* [4]*Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK*

*Email address for correspondence: ruxandra.mutihac@ndcn.ox.ac.uk*

*Keywords: calcium signalling, C9ORF72, motor neurons*

**Background:** An expanded hexanucleotide (GGGGCC)n repeat in chromosome 9 open reading frame 72 (*C9orf72*) has been identified as a major cause of familial amyotrophic lateral sclerosis (fALS) and frontotemporal lobar dementia (FTLD). The expansion is located in an intronic or promoter region upstream of the *C9orf72* coding region and the number of GGGGCC hexanucleotide repeats varies between 100 and 4000 in patients. The function of the *C9orf72* gene and its pathogenic mechanisms are currently unknown.

**Objectives:** The goal of this study is to characterize functional deficits associated with the *C9orf72* hexanucleotide expansions in patient iPSC-derived motor neurons.

**Methods:** Fibroblasts were obtained from healthy subjects and three ALS patients carrying ~500 and ~1000 GGGGCC hexanucleotide repeats in the *C9orf72* gene. Pluripotency was induced by reprogramming the fibroblasts with Sendai viruses carrying Sox2, Oct3/4, Klf4 and c-myc. Embryoid bodies were generated and neuralization was induced by retinoic acid (RA) followed by ventralization, which was achieved by sonic hedgehog agonists. Motor neuron precursors were allowed to reach maturation for 4/6 weeks before functional assays were performed. Functionality was assessed by electrophysiology and live calcium imaging.

**Results:** We found a novel pathogenic link between *C9orf72* mutations and $Ca^{2+}$ signalling dysregulation in ALS iPSC-derived motor neurons. Thapsigargin-evoked $Ca^{2+}$ measurements showed significantly increased $Ca^{2+}$ levels in the endoplasmic reticulum (ER) of *C9orf72* motor neurons. These results correlated with elevated ER stress in the motor neurons derived from *C9orf72* iPSCs. We detected significantly high frequency of PABP+ stress granules, indicating potential autophagy impairments and protein aggregation. Elevated susceptibility to cell death was found in *C9orf72* motor neurons, which showed reduced levels of the anti-apoptotic protein Bcl-2 and increased levels of the apoptotic marker cleaved caspase-3. We also detected characteristic RNA foci in the *C9orf72* motor neurons.

**Discussion and conclusion:** Our cellular model of *C9orf72* iPSC-derived motor neurons reveals disease-specific $Ca^{2+}$ dysregulation which associates with ER stress and increased susceptibility to apoptosis. The *C9orf72* iPSC-derived motor neurons will be a valuable tool for future drug screening and developing improved therapies.

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## C27 THE ROLE OF RBM45 IN ANTIOXIDANT RESPONSES IN ALS

BAKKAR N, KOUSARI A, BOWSER R

*Barrow Neurological Institute, Phoenix, AZ, USA*

*Email address for correspondence: nadine.bakkar@dignityhealth.org*

*Keywords: RBM45, oxidative stress, NRF2*

**Background**: Oxidative stress is a major contributory factor to ALS pathology (1). RNA binding proteins FUS and TDP-43 have been implicated in disease aetiology, although their exact role in pathogenic mechanisms remains unclear. Our group has recently described the involvement of a new RNA-binding protein, RBM45 in ALS (2). Increased levels of RBM45 were detected in the cerebrospinal fluid of ALS

patients, and the protein was localized to cytoplasmic inclusions that often co-localized with TDP43-and ubiquitin positive aggregates.

**Objectives**: In this study, we characterize RBM45 function, subcellular distribution and involvement in the oxidative pathway using various neuroblastoma cell lines (Neuro2a and SH-SY5Y) and rat primary motor neurons. We examine RBM45 binding to various members of the oxidative machinery, and probe the functional consequences of altered RBM45 levels in cellular responses to oxidative stress. In addition, we study the binding of RBM45 to oxidative pathway members in ALS patient lumbar spinal cord homogenates using immunoprecipitation (9 ALS samples and 4 controls) and co-localization approaches.

**Results**: We found that RBM45 binds and stabilizes KEAP, the inhibitor of the antioxidant response transcription factor NRF2 in cells and spinal cord homogenates. Overexpression of RBM45 increases KEAP levels, inhibiting NRF2 and the antioxidant response element signalling pathway, thus increasing cellular death in response to oxidative insult. We further mapped the functional region of the protein responsible for such effects. In addition, we find increased binding of KEAP to RBM45 in ALS patient spinal cord samples as compared to controls.

**Discussion and conclusion**: Our findings define a novel role for RBM45 in the regulation of the oxidative status of the cell. Deregulation of the oxidative pathway has been thoroughly described in ALS and is accepted to be a contributor of neuronal death, yet little is known about the underlying mechanism of this deregulation. Our results show a detrimental effect of RBM45 on cellular response to oxidative injury through interfering with regulators of the antioxidant response *in vitro* as well as *in vivo*. These results provide the first link between an RNA binding protein that can form cytoplasmic inclusions and the KEAP/NRF2/antioxidant response element, signalling pathway in ALS.

**Acknowledgements**: Funding support by NS061867 and NS068179 to RB.

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## C28 HEXANUCLEOTIDE REPEAT EXPANSIONS IN C9ORF72 INDUCE NUCLEOLAR STRESS AND DNA DAMAGE IN NEURONAL CELL LINES

FARG M[1], SULTANA J[1,2], SOO KY[1], ATKIN JD[1,2]

[1]*La Trobe Institute of Molecular Science, Melbourne, Australia,* [2]*School of Advanced Medicine, Macquarie, Sydney, Australia*

*Email address for correspondence: m.farg@latrobe.edu.au*

*Keywords: C9ORF72, hnRNPs, nucleolar stress*

**Background:** Hexanucleotide (GGGGCC) repeat expansions in a noncoding region of C9ORF72 are the major cause of familial ALS (~40%) and FTD (~20%) worldwide. However, how mutations in C9ORF72 lead to neurodegeneration in ALS and FTD is unclear. The repeat expansion forms RNA foci that may sequester RNA binding proteins, leading to RNA dysfunction and cell death. We and other researchers recently demonstrated that C9ORF72 interacts with heterogeneous nuclear ribonucleoproteins (hnRNP) hnRNPA2/B1 and hnRNP A1, which become activated and shuttle to the cytoplasm upon nucleolar stress. Here we used the nucleolar stress marker, B23, a multifunctional chaperone that translocates from the nucleolus after DNA damage. B23 activates phosphatidylinositol 3-kinase (PI3K) and its downstream effector, serine/threonine kinase (Akt). Both of these pathways affect multiple cellular functions including DNA repair, proliferation, and cell survival. Impairment of ribosome biogenesis and nucleolar stress leads to p53 induction and cell cycle arrest.

**Objective:** To identify mechanisms by which the repeat expansion in C9ORF72 triggers ALS and FTD. Markers of both the PI3K-AKT-MTOR signalling pathway and nucleolar stress were examined in neuronal cell lines transfected with constructs encoding either three or thirty GGGGCC repeats linked to GFP or control GFP only. Activation of stress granules, hnRNPs and cellular markers involved in cell growth, proliferation and survival processes, were also examined in these cells.

**Methods:** Immunocytochemistry and immunoblotting of cell lysates from SH-SY5Y neuroblastoma cells transfected with either $(GGGGCC)_{3}$ or $(GGGGCC)_{30}$ constructs linked to GFP was performed using antibodies against specific markers linked to cell survival. This included the translation initiation factor 4E binding protein 1 (4E-BP1), S6 kinase, phospho-AKt, elongation factors eIF4G-and eIF4E, p53 and H2AX, which detects double-stranded breaks in DNA and initiates cell death in response to DNA damage.

**Results:** Expression of $(GGGGCC)_{30}$-GFP formed intranuclear inclusions and cytoplasmic activation of hnRNP A2/B1 and hnRNP A1 in SH-SY5Y cells. The levels of B23 were significantly reduced in cells expressing $(GGGGCC)_{30}$-GFP compared to controls, providing evidence of nucleolar stress. Also, heat shock proteins hsp90 and hsp70, pro-apoptotic p53 and phosphorylated H2AXδ were up-regulated in cells expressing $(GGGGCC)_{30}$-GFP.

**Discussion and conclusion:** Dysregulation of neuronal RNA and the formation of stress granules are associated with many neurodegenerative diseases including ALS. However our data demonstrate that the GGGGCC repeat expansion in C9ORF72 also triggers DNA damage, which may affect transcription and RNA splicing. Also we detected evidence of nucleolar stress. Furthermore, the up-regulation of H2AX and p53 link these mechanisms to apoptotic cell death. Our findings therefore imply that dysfunction to the nucleolus may trigger neurodegeneration in ALS.

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# SESSION 5B CARE PRACTICE

## C29 THE ROLE OF EHEALTH IN ALS – THE DIGITAL AGENDA

MEYER T, MÜNCH C

*Charité – Universitätsmedizin, Berlin, Germany*

*Email address for correspondence: thomas.meyer@charite.de*

*Keywords: care provision, digitalisation, eHealth*

The majority of our data on ALS care comes from clinical studies and trials. There are few systematic 'real life' data on the utilisation of clinical pathways, guidelines and outcomes in ALS. Data on costs are at hand mostly from insurance companies and healthcare plans. In the face of this lack of ALS health care data, only rudimentary tools for quality management and benchmarking have been established. However, there is a 'mega trend' for the use of the internet and electronic media in medicine (eHealth). This process of change has the potential to improve ALS care provision. With digitalisation, three lines of action are anticipated which require our active involvement:

1.1.Digitalisation of information: The electronic capture of data and the internet facilitate three major developments: i) new ways of capturing data (eg, electronic health records); ii) remote access to care data (telemedicine); iii) new types of data (such as patient reported outcomes).

1.2.Digitalisation of processes: the coordination and logistics of ALS care is complex such as in nutrition management, home ventilation, and palliative drug therapy. The 'Internet of Services' facilitates the linking of all the key players involved as well as of necessary information.

1.3.Digitalisation of medical products and devices: Major progress is made with the recombination of information technology and 'traditional' assistive devices such as wheelchairs, communication systems, but also PEG pumps and respirators. Medical devices in ALS are becoming more mobile, smarter and interactive. We are entering the 'Internet of Things'.

At the same time, digitalisation confronts us with new challenges that are largely unmet and have to be addressed:

2.1.Data safety and legal issues: technical and legal conditions for the medical use of the internet are far from being harmonized within most countries and even less so at an international level.

2.2.Access to digital services for patients: patients and ALS professionals face technical, social and emotional barriers to the use of digital services.

2.3.Costs of digital infrastructure and services: the issues of digitalisation of information (electronic health record) and processes (digital services) as well as the reimbursement to ALS professionals for digital interaction are largely unresolved and represent a major barrier to innovation.

These numerous tasks are to be met in an evolutionary process. However, the chances of digitalization overweight the risks and challenges. Digitalization includes the chance to make ALS care more accessible, interactive, patient-centred, faster and safer. ALS professionals and patients alike ought to take an active and creative role in the implementation of the digital agenda and the delivery of the tasks ahead.

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## C30 THE IMPACT OF NECK WEAKNESS AND EXPERIENCES OF USING NECK ORTHOSES IN PEOPLE WITH MOTOR NEURONE DISEASE

MCDERMOTT CJ[1], BAXTER S[1], REED H[2], CLARKE Z[3], HERON N[4], LANGLEY J[2], QUINN A[5], SQUIRE G[1], STANTON A[2], SHAW PJ[1]

[1]*University of Sheffield, Sheffield, UK,* [2]*Sheffield Hallam University, Sheffield, UK,* [3]*Barnsley Hospitals NHS Foundation Trust, Barnsley, UK,* [4]*Sheffield Teaching Hospitals, Sheffield, UK,* [5]*Yorkshire MND Association, Sheffield, UK*

*Email address for correspondence: s.k.baxter@sheffield.ac.uk*

*Keywords: neck weakness, orthoses, quality of life*

**Background:** Patients with motor neurone disease experience weakness affecting muscles, including those of the neck. A review of available neck supports however concluded that they did not satisfy the needs of people with motor neurone disease and patients may often abandon use of an unsatisfactory device (1).

**Objectives:** The data presented here forms part of a wider research project that aims to produce a new neck orthosis. The aims of this part of the study were: 1) to examine perceptions of supports currently in use; and 2) to describe the impact of neck weakness on people living with motor neurone disease.

**Methods:** The study used a mixed method design, collecting quantitative data in the form of a questionnaire rating elements of design and usage, and qualitative data obtained during interviews exploring views of supports that had been tried, and how neck weakness affected every day functioning.

**Results:** Twenty six patients were recruited to the study. A range of supports were being used by participants, with foam collars and the Head Master the most frequently described. There was variation between participants regarding the supports that had been available to them. Orthoses were described as: being difficult to fit; providing little support; being overly restrictive; being uncomfortable; and being generally unsuitable for their needs. Some positive comments regarding supports were also outlined. Participants described how neck weakness impacted on eating and management of saliva, with some experiencing ongoing pain and discomfort. Also, patients and carers described how a lower eye level affected social interaction and activities of daily living. Other key areas of adverse impact were travelling and general mobility. Analysis of the scaled questionnaire data indicated that while participants reported that collars tended not to restrict breathing, there could be difficulties eating and drinking and there were reports of orthoses causing frustration. The appearance of the collars and ease of fitting was rated poorly, and patients generally disagreed that they were satisfied with the product.

**Discussion and conclusion:** The findings highlight the seemingly often unplanned and variable provision of neck support for patients. Participants described the considerable impact on life that neck weakness had on everyday functioning, suggesting that neck support should be viewed as a priority area within care for people with MND. The limitations of currently available orthoses however hinder provision of

suitable supports. Patients may be left with a choice of either using no orthosis or accepting a non-ideal device.

**Acknowledgements:** This project was funded by the National Institute for Health Research Innovation for Invention Programme.

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## C31 A DESCRIPTION OF PAIN IN ALS

STEPHENS H[1], WALSH S[2], SIMMONS Z[1]

[1]*Penn State Hershey Medical Center, Hershey, PA, USA,* [2]*ALS Association, Greater Philadelphia Chapter, Harrisburg, PA, USA*

*Email address for correspondence: hstephens1@psu.edu*

*Keywords: pain, symptom management, treatment*

**Background:** ALS has generally been considered a painless disorder, but recent studies have shown that pain is a frequently underestimated and under-reported symptom in ALS, more so in the late stages of the disease with a prevalence of pain of up to 70% at some time during the course of the disease. Minimal research exists to describe the experience of pain in ALS.

**Objective:** To describe pain in ALS.

**Methods:** An electronic survey including demographics, ALS Functional Rating Scale-R (ALSFRSR), and the Brief Pain Inventory-Long Form (BPI) was sent to 319 registered patients of the ALS Association Greater Philadelphia Chapter who have subscribed to email communications from the Chapter. All participants completed demographics and ALSFRSR. Only participants who responded that they had pain proceeded in the survey to complete the BPI. The study received exempt IRB approval. Descriptive statistics and qualitative analysis were utilized to examine the data.

**Results:** A 27% response rate was obtained: 87 participants participated in the survey and 56% reported pain. Sixty-eight percent of the respondents were men; mean age 60 years; onset 14% bulbar, 70% limb, 4% respiratory, and 12% generalized. Mean disease duration was 60 months (sd = 64 months, median 33 months, range = 1 to 304 months). Mean ALSFRSR was 30.6, sd = 9.6, range = 8–48. There were no significant differences in the composition of participants reporting pain vs. no pain. 53% reported no other painful condition before ALS. Pain was present at ALS onset in 28% of respondents, with the most frequent sites of pain being the neck, shoulders, and proximal limbs. Average pain severity score was 3.4/10, sd = 1.91. Average pain interference with daily life score was 3.9/10, sd = 2.9. Frequent descriptors of pain included aching (78%), sharp (62%), tiring (63%), and nagging (54.3%). 18% use no pain medicine. 48% take pain medications only when necessary and 34% on a regular basis. 20% reported that they need a stronger pain medicine and 22% were uncertain. The majority of respondents were not concerned about overuse of pain medication, while 26% were, and 7% remained uncertain. Alternative pain relief methods utilized by the sample included relaxation techniques (47%), warm compresses (41%) and distraction (39%). On average, participants reports 60% relief from pain using medication and other treatments.

**Discussion and conclusion:** Pain is a significant component of ALS. Over half of the participants reported pain which is, on average, moderate in severity and interference with daily life. Over 80% of respondents were using pain medication. While the majority of participants were satisfied with their pain control, one fifth wished for stronger medication and one fifth were unsure about their treatment needs. A small portion of participants were concerned about overuse of pain medication. In general, non-pharmacologic treatments were utilized for pain relief.

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## C32 ADVANCE CARE PLANNING IN A DUTCH TERTIARY ALS CENTRE, AN EVALUATION OF A DUTCH CARE APPROACH

SEEBER A[1,2], POLS J[2], HIJDRA A[1], GRUPSTRA H[3], WILLEMS D[2], DE VISSER M[1]

[1]*Department of Neurology,* [2]*Section of Medical Ethics, Department of General Practice,* [3]*Department of Rehabilitation, Academic Medical Center, Amsterdam, The Netherlands*

*Email address for correspondence: a.a.seeber@amc.uva.nl*

*Keywords: advance care planning, patient empowerment, quality of life*

**Background:** Many neurological diseases are relentlessly progressive and incurable. There is increasing awareness of the need for an integrated approach to patient palliative care including advance care planning (ACP) which should assure patients' participation in decision-making before they become cognitively and communicatively incapable to do so. However, in daily practice discussions about future care options and preferences remain scarce as they are experienced as difficult for both physicians and patients. Interestingly, in the Netherlands tertiary ALS centre (NAC), early discussions about and planning of future medical care is common practice.

**Objective:** To (1) study the actual timing and content of discussions about future care during the outpatient clinic's office hours of the NAC and (2) learn how patients with a motor neuron disease experience this practice.

**Methods:** We performed non-participating observations in all appointments that patients with ALS and PMA had with their own ALS specialist during 6 consecutive months, and carried out in-depth interviews with these patients about their experiences with the ACP approach. Qualitative analysis consisted of open coding, followed by inductive analysis of all written material, observation reports and verbatim typed out interviews.

**Results:** 28 patients were followed from the outpatient clinic during 6 consecutive months. 21 of them were subsequently interviewed in-depth. Patients varied in age, sex, disease onset, symptoms and rapidity of physical decline. All patients were born and raised in the Netherlands. The actual timing of discussions about future care options was closely linked to the kind of information which was discussed. Shortly after diagnosis the specialist gave a rather general outlook upon the future with progressive physical decline, care needs and options, provided the patient did not yet experience serious physical restraints. As more concrete disease-related problems became apparent, the more detailed information was offered. The patients appreciated this policy of stepwise and repeated discussions - as part of the MND specialist's professional guidance throughout the illness trajectory.

**Discussion:** Our study shows that patients with ALS and PMA appreciate ACP, as an integrated part of long-term follow-up. The specialists' strategy of 'setting the agenda' for the next appointment(s), an agenda which is based on disease-specific and in due course on patient-specific care needs and preferences, appears to facilitate initiation and maintenance of discussions about future and end-of-life care issues. In this context, living wills and do-not-resuscitate orders are rather a tool to pursue discussing life perspectives than the ultimate goal of ACP.

**Conclusion:** Advanced care planning is feasible for both ALS specialists and their patients. ACP facilitates customized care and could become a template for long-term specialized care of patients with other progressive and incurable neurological diseases.

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# SESSION 6A CELL BIOLOGY AND PATHOLOGY

## C33 FUNCTIONAL AND STRUCTURAL CHARACTERIZATION OF THE HNRNP TDP 43 AND ITS INTERACTIONS

BARALLE F

*ICGEB, Trieste, Italy*

*Email address for correspondence: baralle@icgeb.org*

*Keywords: TDP-43, ALS pathogenesis, aggregation*

Nuclear factor TDP-43 is a multifunctional RNA binding protein that is involved in cellular processes, such as RNA splicing stability and transport, which are essential for the correct maintenance of neuronal activity and survival. TDP-43 is the major protein component of the cytoplasmic insoluble aggregates found in affected neurons in neurodegenerative diseases such as FTLD and ALS.

We have carried out detailed studies of TDP-43 structure, mapped the amino acid sequences essential for the RNA binding function of this protein and those responsible for the protein-protein interactions involved in its biological activities. We have shown that TDP-43 controls its own cellular levels by a negative feedback loop that involves binding to the 3'UTR of its own transcript. The binding of excess TDP-43 molecules to the 3'UTR triggers a novel mechanism of RNA degradation that does not involve NMD but is based on interactions between an unproductive spliceosome complex and the poly A synthesis machinery. This process is conserved through evolution.

We have also focused on mapping the regions responsible for TDP-43 aggregation and established that a region rich in Q/N repeats localized between residues 321–366 of the C-terminal domain of TDP-43 can induce aggregation when overexpressed in a variety of human and mouse cell lines and in *Drosophila melanogaster*. This region is also responsible for the interaction between TDP-43 and other hnRNP proteins in all species studied from man to fly. This fact suggests a rich variety of pathways essential for the homeostasis of TDP-43 levels and maintenance of function. We have used the 321–366 amino acid sequence to build a model of aggregation that resembles as closely as possible what is observed in the aggregates found at the end point of the human pathological process. We have shown that aggregation sequesters TDP-43 altering the levels of functional protein and hence affecting its role in the splicing of endogenous genes. The self-regulation loop and the effect of aggregation converge in these findings. In fact nuclear or cytoplasmic aggregation of TDP-43 may break the self-regulatory cycle by sequestering functional protein and hence stimulate an increase of TDP-43 production. If aggregates grow and sequester TDP-43 beyond the nuclear capacity to produce it, changes in the splicing of endogenous genes become evident, indicating a loss of function effect. We have shown in Drosophila that this process leads also to locomotion defects.

We are now screening small molecule effectors for their potential to prevent or revert aggregation, restoring TDP-43 functionality in the cell.

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## C34 FUTSCH/MAP1B IS A TRANSLATIONAL TARGET OF TDP-43 AND MITIGATES TOXICITY IN MOTOR NEURONS

COYNE A[1], BAGEVALU SIDDEGOWDA B[1], ESTES P[1], JOHANNESMEYER J[1], DANIEL S[1], PEARSON A[1], BOWSER R[2], ZARNESCU D[1]

[1]*University of Arizona, Tucson, USA,* [2]*Barrow Neurological Institute, Phoenix, USA*

*Email address for correspondence: zarnescu@email.arizona.edu*

*Keywords: TDP-43, translation, microtubule stability*

**Background:** Dysregulation of RNA metabolism has recently emerged as a major contributor to the pathophysiology of ALS. TDP-43 is an RNA binding protein linked to ALS that regulates the splicing and transport of specific mRNAs (1). Additionally, TDP-43 associates with RNA stress granules, which in turn can impact translation initiation. Although TDP-43 has been shown to interact with translational regulators, its role in protein synthesis remains unclear and no *in vivo* translational targets have been reported to date.

**Objectives:** To uncover the role of TDP-43 in translation and identify translational targets that mediates its toxicity in motor neurons.

**Methods:** To identify mRNAs that are dysregulated at the level of translation we performed RNA sequencing in conjunction with polysome fractionations of Drosophila expressing TDP-43 in motor neurons. To test the physiological significance of candidate targets we took a combined molecular and genetic approach in Drosophila and used ALS tissues for target validation.

**Results:** We found vastly different dystranslated gene sets in the context of wild-type TDP-43 versus G298S overexpression suggesting that although both variants lead to ALS-like phenotypes, they likely utilize distinct mechanisms of neurotoxicity *in vivo*. Among the mRNAs altered in polysomes we identified *futsch*, the Drosophila homolog of MAP1b, a microtubule stabilizing protein linked to synaptic growth and stability (2). Immunoprecipitation experiments show that *futsch* mRNA associates with TDP-43 in a complex. Quantification of mRNA and protein levels indicates that *futsch* expression is negatively regulated by TDP-43 post-transcriptionally. This is substantiated by qPCR in conjunction with polysome fractionation experiments indicating that, in the context of TDP-43, *futsch* mRNA shifts from actively translating polysomes to non-translating RNPs. Consistent with these findings, *futsch* overexpression extends lifespan and suppresses TDP-43 dependent phenotypes including locomotor dysfunction as well as neuromuscular junction (NMJ) abnormalities linked to microtubule and synaptic stabilization. Furthermore, fractionation experiments indicate that overexpressing *futsch* in motor neurons significantly reduces TDP-43 aggregation. Despite a clear reduction in *futsch*/MAP1B levels at the NMJ, its expression is upregulated in motor neuron cell bodies both in Drosophila and ALS spinal cords consistent with an additional potential defect in axonal transport.

**Discussion and conclusion:** These results demonstrate that *futsch*/*MAP1B* is a translational target of TDP-43 and provide novel insights into microtubule and synaptic stabilization dependent disease mechanisms in ALS.

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## C35 ISOFORM-SPECIFIC ANTIBODIES REVEAL REGION-DEPENDENT CHANGES IN C9ORF72 PROTEIN LEVELS IN BRAINS FROM ALS CASES WITH REPEAT EXPANSIONS IN C9ORF72

MCGOLDRICK P[1], XIAO S[1], MCKEEVER PM[1], MCLEAN JR[1], XI Z[1], KEITH J[2], ROGAEVA E[1], ZINMAN L[2], ROBERTSON J[1]

[1]*Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, Canada,* [2]*Sunnybrook Health Sciences Centre, Toronto, Canada*

*Email address for correspondence: philip.mcgoldrick@utoronto.ca*

*Keywords: C9ORF72, antibodies, haploinsufficiency*

**Background:** A noncoding hexanucleotide repeat expansion in *C9orf72* is the most commonly known cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). It has been reported that the repeat expansion causes a downregulation of *C9orf72* transcripts; leading to the suggestion that haploinsufficiency may contribute to disease pathogenesis. It is predicted that from three transcript variants of *C9orf72*, two protein isoforms are generated through alternative splicing; a long form (C9-L) and a short form (C9-S). Although many groups have assessed levels of *C9orf72* transcripts, there are few reports on the effects of the repeat expansion at the protein level.

**Objectives:** To generate antibodies specifically recognising *C9orf72* protein isoforms, and use these antibodies to characterize the biochemical profile and expression of *C9orf72* in post mortem tissue from *C9orf72* and sporadic ALS patients.

**Methods:** Polyclonal antibodies were generated by immunizing rabbits against peptide sequences of C9-L and C9-S proteins. Antibody specificity was confirmed using constructs encoding tagged C9-L or C9-S proteins. Patients were diagnosed at the ALS Clinic of Sunnybrook Health Sciences Centre in Toronto, using El Escorial Criteria. Consent for autopsy was obtained with approval by local ethical review board. Sequential protein extraction was carried out on frontal cortex and cerebellar tissue, and standard western blot protocols performed.

**Results:** Polyclonal antibodies were generated which identify C9-L and C9-S proteins. Following sequential protein extraction from human brain tissue, we noted distinct biochemical profiles of C9-L and C9-S. Quantification of C9-L levels in frontal cortex tissue showed significantly lower levels in *C9orf72* cases compared to sporadic ALS cases ($n = 8$ per group, $p < 0.05$), and although a similar trend was apparent in cerebellar tissue, this was not significant ($n = 8$ per group). C9-S levels were not significantly different between *C9orf72* and sporadic ALS cases in either region examined ($n = 8$ per group).

**Discussion and conclusion:** We have generated antibodies which specifically recognize *C9orf72* protein isoforms, demonstrated distinct biochemical profiles of the isoforms in ALS brain tissue, and shown that the repeat expansion in *C9orf72* leads to region-specific downregulation of C9-L protein levels, providing support that haploinsufficiency of *C9orf72* is a contributing factor to disease pathogenesis.

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## C36 C9ORF72 EXPANSIONS ARE POTENTIALLY PATHOGENIC ON THREE BIOLOGICAL LEVELS

BOEYNAEMS S[1,2], BENTO-ABREU A[1,2], BOGAERT E[1,2], VANDERLINDEN W[3], BRULLOT W[4], TIMMERS M[1,2], NUYTS R[1,2], SCHEVENEELS W[1,2], VERBIEST T[4], DE FEYTER S[3], VAN DAMME P[1,2], VAN DEN BOSCH L[1,2], ROBBERECHT W[1,2]

[1]*Lab of Neurobiology, Vesalius Research Center, VIB, Leuven, Belgium,* [2]*Experimental Neurology,* [3]*Laboratory of Photochemistry and Spectroscopy, Department of Chemistry,* [4]*Laboratory for Molecular Electronics and Photonics, Department of Chemistry, KU Leuven, Leuven, Belgium*

*Email address for correspondence: steven.boeynaems@vib-kuleuven.be*

*Keywords: C9ORF72, RAN translation, RNA toxicity*

**Background:** GGGGCC tandem repeat expansions in the C9ORF72 gene are the most prevalent mutations underlying familial ALS/FTLD. However, the pathogenic mechanism through which they induce neurodegeneration remains unknown. Different non-exclusive mechanisms might be at work and include loss- and gain-of-functions of the mutant C9ORF72 allele, at the DNA, RNA and protein level.

**Objectives:** Unraveling the complexity of C9ORF72 ALS/FTLD using an *in vivo* zebrafish model.

**Results:** Using a zebrafish model we found suggestive evidence for a multifaceted pathogenesis: (1) knock-down of the fish endogenous C9ORF72 homologue induced an axonal phenotype, reminiscent of what is seen in mutant SOD1 and TDP-43 fish models. We, and others have gathered evidence that lowered transcription in patients can be explained through secondary structures of the DNA repeat; (2) overexpression of repeat RNA induced similar axonal phenotypes in the fish. Moreover, using structural biology, such as single molecule microscopy, we were able to link this toxicity to the formation of specific secondary structures. Importantly, we could exclude that this RNA toxicity was mediated by RAN translation; (3) to investigate a potential contribution of aggregating DPRs to disease we investigated their toxicity by ruling out any confounding RNA effects.

**Conclusion:** Our data, generated using an *in vivo* vertebrate model, provides evidence that C9ORF72 repeat expansions can induce toxicity through different pathogenic mechanisms.

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## C37 RNA-DEPENDENT AND RNA-INDEPENDENT AGGREGATION OF FUS IN THE CELL CYTOPLASM: WHAT STRUCTURES BECOME PRECURSORS OF PATHOLOGICAL INCLUSIONS IN FUSOPATHIES?

SHELKOVNIKOVA T[1,2], ROBINSON H[1], OVCHINNIKOV R[2], NINKINA N[2,3], BUCHMAN V[1]

[1]*School of Biosciences, Cardiff University, Cardiff, Wales, UK,* [2]*Institute of Physiologically Active Compounds Russian Academy of Sciences, Chernogolovka, Moscow Region, Russia,* [3]*Institute of General Pathology and Pathophysiology of Russian Academy of Medical Science, Moscow, Russia*

*Email address for correspondence: buchmanvl@cf.ac.uk*

*Keywords: FUS/TLS, protein aggregation, RNA granules*

**Background:** A number of RNA-binding proteins have been recently implicated in aetiology and pathogenesis of ALS and related diseases. These proteins are important normal constituents of various cytoplasmic and/or nuclear RNA granules and their reversible aggregation is believed to be involved in formation of at least some of these granules. In the disease-affected cells these proteins aggregate irreversibly and consequently, give raise to various deposits or inclusions. It has been suggested that physiological RNA granules or RNP complexes forming in stressed cells (eg, stress granules) might become precursors of such pathological structures.

**Objectives:** We studied mechanism of aggregation of cytoplasmically mislocalised FUS as a prototypical RNA-binding protein that forms pathological aggregates in neurons of patients with certain forms of ALS and FTD.

**Methods:** Aggregation of FUS and formation of characteristic cytoplasmic profiles were studied in cultured cells and transgenic mice expressing various isoforms of human FUS using biochemical, cytological and immunochemical techniques.

**Results:** Mislocalised FUS variants lacking major RNA binding domains (either RGG and Zn-finger or RRM) aggregate quickly and efficiently in the cytoplasm of cultured cell and neurons of transgenic mice. This irreversible pathological aggregation is distinct from RNA-dependent reversible aggregation important for physiological function(s) of FUS. Studies of cells expressing FUS variants found in association with familial ALS and capable of RNA binding demonstrated that they reversibly aggregate via RNA-dependent two-step mechanism and form unusual types of cytoplasmic RNP granules that structurally 'mimic' although these are not identical to physiological transport RNA granules or stress granules. In conditions of RNA deficiency these pseudo-physiological RNA-dependent granules undergo transformation into RNA-free aggregates similar to those formed by variants lacking the ability to bind RNA.

**Discussion and conclusion:** We propose that a multistep process of pathological FUS aggregation in the cell cytoplasm involves RNA-dependent and RNA-independent mechanisms. A similar cascade of molecular events might be involved in triggering FUSopathy and potentially, other RNPopathies.

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## C38 ELP3 AS A DISEASE MODIFIER IN ALS

BENTO-ABREU A, TIMMERS M, ROBBERECHT W

*V.I.B. - K.U.Leuven, Leuven, Belgium*

*Email address for correspondence: andre.bentoabreu@vib-kuleuven.be*

*Keywords: elongator, neuroprotection, SAM domain*

**Background:** ELP3 is member of the Elongator complex, comprised of six subunits (ELP1-ELP6). Elongator was discovered in yeast, where it associates with the hyperphosphorylated carboxy-terminal domain of RNA polymerase II, and regulates transcription elongation. Moreover, ELP3 is involved in tRNA wobble modifications, being required for the side chain formation of uridines at position 34, necessary to increase translation efficiency. We have identified a polymorphism in the ELP3 gene that is associated with ALS. Lower expression levels of Elp3 were found in the brain of individuals with the ALS at-risk genotype. Moreover, two loss-of-function mutations in the drosophila ELP3 were identified to induce profound axonal and synaptic defects, and the knockdown of Elp3 in zebrafish induced motor axonal abnormalities.

**Objectives:** To assess whether ELP3 is a modulator gene in ALS.

**Methods:** Overexpression and knock-down of Elp3 in the $SOD1^{G93A}$ mouse model of ALS and also in the SOD1A4V zebrafish model.

**Results:** The $ELP3^{-/-}$ mouse is embryonically lethal at E10.5. Nonetheless, $ELP3^{-/-}$ are viable and $ELP3^{+/-}$ $SOD1^{G93A}$ mice become symptomatic earlier than $SOD1^{G93A}$ mice ($98.7 \pm 2.2$ days vs. $105.8 \pm 2.1$ days), although the survival of these mice is not affected. Surprisingly, knock-down of Elp3 (90%) in adult mice leads to death within 40 days. We are currently investigating this. On the other hand, Elp3 overexpression in adult mice (60 days-old) delays the onset of the disease and prolongs the survival of $SOD1^{G93A}$ mice by 9 days (153 days vs. 162 days). These results confirm previous data from AAV9-mediated overexpression of Elp3 in the spinal cord of $SOD1^{G93A}$ mice, where the survival was extended by 9 days (145.9 days (AAV9: GFP) vs. 158.5 days (AAV9: Elp3)). In the zebrafish, the motor axonopathy induced by Elp3 knockdown or SOD1 A4V expression is rescued by wild-type Elp3 and also by two HAT domain mutants, but not by SAM domain mutants.

**Discussion:** Elp3 overexpression is beneficial, both in the zebrafish (rescue of SOD1-induced axonopathy) and in an ALS mouse model (increasing it lifespan), whereas Elp3 reduction is detrimental, both in an ALS model (earlier onset of symptoms) and in the zebrafish (inducing axonopathy). The potential role of Elp3 in neuroprotection is, apparently, independent of acetylation. The SAM domain is involved in methylation/demethylation reactions. It is reasonable to speculate that ELP3 might regulate the translation of certain stress-induced proteins via tRNA wobble modifications. Further investigation is needed to clarify the role of ELP3 in ALS.

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# SESSION 6B EPIDEMIOLOGY

## C39 GENETICS AND PHENOTYPES OF AMYOTROPHIC LATERAL SCLEROSIS IN MAINLAND CHINA

CUI L

*Peking Union Medical College Hospital, Beijing, China*

*Email address for correspondence: pumchcuily@sina.com*

*Keywords: gene, phenotype, treatment*

**Objective:** To determine the distribution of the most commonly mutated genes, and genotype/phenotype associations in Chinese ALS patients.

**Methods:** A registered study of ALS patients was conducted across 10 hospitals in 7 Chinese cities and a systematic review of research findings of SOD1, TARDBP, FUS, C9orf72 and other gene mutations was conducted in Chinese publications.

**Result:** It was found that the mean age at onset of ALS in China is 52.4 ± 12.1 years, which is earlier than in many developed countries (1). The mean duration from onset to diagnosis was 13.8 ± 10.1 months. The male to female ratio was 1.63:1.Ten patients had family history of ALS/MND (2.7%).

Across all of the 455 patients, 182 (40%) were professional workers; 233 (51.2%) were manual labourers and 40 (8.8%) were from unknown occupations. Seventy-one (15.6%) patients had a history of toxic substance abuse. 34.5% patients would accept non-invasive ventilation, and 18% would accept mechanical ventilation. Riluzole treatment was used in 133 patients (29.2%). 176 patients (38.7%) had used traditional herbal treatments (2).

Analysis showed that SOD1, FUS, TARDBP and C9orf72 gene are the most common gene mutations in Chinese FALS patients (26.15%, 12.5%, 5.6% and 1.1%) and in SALS patients (1.61%, 1.56%, 0.52 and 0.3%). The most frequently mutated gene is the SOD1 gene with mutations at C6, C16, V29, H46 and L84 being the most prevalent in Chinese ALS populations (3–13). The most common mutation in the TARDBP gene is the S292N gene mutation.

**Conclusions**: Our analysis showed the presence of population differences, whereby common gene mutations are different among Chinese, Asian, Europe and the United States populations.

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## C40 MILITARY SERVICE AND AMYOTROPHIC LATERAL SCLEROSIS IN A POPULATION-BASED COHORT

WEISSKOPF M[1], CUDKOWICZ M[2], JOHNSON N[3]

[1]*Harvard School of Public Health, Boston, MA, USA,* [2]*Massachusetts General Hospital, Boston, MA, USA,* [3]*U.S. Census Bureau, Suitland, MD, USA*

*Email address for correspondence: mweissko@hsph.harvard.edu*

*Keywords: epidemiology, military, risk factors*

**Background:** Service in the U.S. military has been associated with a higher risk of ALS. Only one study has examined this for military service prior to the Gulf War, and none have examined a U.S. representative population.

**Objective:** To examine the association between military service and the risk of ALS in a U.S. population-representative cohort.

**Methods:** We prospectively assessed the relation between service in the military and ALS mortality among participants in the National Longitudinal Mortality Study, a U.S. population-representative cohort of U.S. men and women surveyed from 1973 through 2002. Participant follow-up for cause of death was conducted from 1979 through 2002 for ALS mortality. There were 696,743 men and 35,227 women 25 years and older with military service data. In this group there were 375 male and 96 female ALS deaths. Adjusted hazard ratios (HRs) were calculated using Cox proportional hazards.

**Results:** Men who served in the military had an increased death rate from ALS (HR: 1.21; 95% CI: 0.97–1.50) compared with those who did not serve. An increase in ALS mortality was found among those who served during World War II (HR: 1.46; 95% CI: 1.13–1.88), but not from other time periods. This pattern was similar for women, but with larger confidence intervals (HR for military service: 1.37; 95% CI: 0.34–5.57; HR for service during World War II: 2.15; 95% CI: 0.52–8.85).

**Discussion and conclusion:** Military personnel have an increased risk of ALS, which may be specific to certain service periods. Because of the longer follow-up time for World War II veterans, we cannot rule out that increased risk for those who served during other periods would be seen with further follow-up.

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## C41 ASSOCIATION BETWEEN PREMORBID DIABETES MELLITUS AND RISK OF AMYOTROPHIC LATERAL SCLEROSIS IN THE SWEDISH POPULATION

MARIOSA D[1], KAMEL F[2], BELLOCCO R[1,3], YE W[1], FANG F[1]

[1]*Karolinska Institutet, Stockholm, Sweden,* [2]*National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA,* [3]*University of Milano-Bicocca, Milan, Italy*

*Email address for correspondence: daniela.mariosa@ki.se*

*Keywords: metabolism, diabetes mellitus, insulin dependence*

**Background:** Energy metabolism is altered in patients with amyotrophic lateral sclerosis (ALS). Less is known about the characteristics of this association, including the temporal relationship of diabetes and ALS, and the role of insulin-dependence.

**Objectives:** To examine whether pre-existing diabetes was associated with a lower subsequent risk of ALS and to evaluate the potential impact of insulin-dependence and diabetes duration on such an association.

**Methods:** We conducted a population-based case-control study of 5,108 ALS cases and 25,540 individually matched population controls during 1991–2010. Information on ALS and pre-existing diabetes was retrieved from the nationwide Swedish Patient Register. Multivariable conditional logistic regression modelling was used to explore the association of ALS with any type of diabetes overall, and with insulin-dependent or non-insulin dependent diabetes specifically. Variation of the association with diabetes duration and by age at ALS diagnosis, gender and length of disease was also studied.

**Results:** In total, 224 ALS cases (4.39%) had been diagnosed with diabetes before the index date compared to 1,437 controls (5.63%), leading to an overall inverse association between diabetes and ALS risk (OR = 0.79, 95% CI 0.68–0.91). The inverse association was noted for non-insulin-dependent diabetes (OR = 0.66, 95% CI 0.53–0.81) but not for insulin-dependent diabetes (OR = 0.83, 95% CI 0.60–1.15). The protective effect of diabetes on ALS varied as a function of diabetes duration, with the strongest association observed around six years after first confirmation of diabetes in the Patient Register. Furthermore, the association was strongly age-specific; the inverse association was noted only among individuals age 70 or older. For younger individuals (< 50 years), pre-existing insulin-dependent diabetes was associated with a higher risk of ALS (OR = 5.38, 95% CI 1.87–15.51).

**Discussion and conclusion:** Our study provided important evidence for an association between premorbid diabetes and ALS, highlighting the importance of taking into account age, insulin dependence and diabetes duration when examining such an association.

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## C42 HEAD INJURY DOES NOT ALTER DISEASE PROGRESSION OR NEUROPATHOLOGIC OUTCOMES IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

FOURNIER C, POLAK M, BORDEAU J, MCSHANE L, SHAW L, GLASS J

*Emory University, Atlanta, GA, USA*

*Email address for correspondence: christina.nicole.fournier@emory.edu*

*Keywords: head injury, tau, TDP-43*

**Background:** Head injury has been examined as a risk factor for ALS (1). A causal relationship between head injury and ALS has been proposed after observing pathologic findings of tau proteinopathy (the pathologic finding described with chronic traumatic encephalopathy) and TDP-43 accumulation in the brain in professional athletes with ALS (2).

**Methods:** ALS patients were surveyed to obtain head injury history, and clinical information was obtained from medical records. Head injury was defined as an event associated with loss of consciousness or requiring hospitalization, occurring greater than 1 year prior to ALS diagnosis. Demographic and clinical information from ALS patients with head injury was compared to ALS patients without. Linear regression was performed with head injury as a predictor variable and mean monthly ALSFRS-R decline as the outcome while controlling for potential confounders.

Additionally, head injury history was obtained from family members of ALS autopsy cases. The frequency of tau proteinopathy, TDP-43 proteinopathy in the brain, and Alzheimer dementia (AD) pathology were examined comparing ALS cases with head injury, to ALS cases without. Logistic regression was performed with each independent neuropathologic diagnosis as an outcome measure and head injury as a predictor.

**Results:** No difference was seen in the rate of decline of ALSFRS-R between ALS patients with (n = 24) and without (n = 76) head injury, with mean monthly decline of ALS-FRS-R of -0.9 for both groups. Head injury (p = 0.18), participation in athletics (p = 0.34), military service (p = 0.20), and smoking (p = 0.06) were not significant predictors ALS-FRS-R mean monthly decline.

Of 47 autopsy cases (n = 9 with head injury; n = 38 without), no significant differences were seen in the frequency of tau proteinopathy (11% of head injury cases; 24% of cases without), TDP-43 proteinopathy in the brain (44% of head injury cases; 45% of cases without), or pathologic findings of AD (33% of head injury cases; 26% of cases without). Independent logistic regression models showed that head injury was not a significant predictor of tau pathology (OR = 0.4, p = 0.42) or TDP-43 pathology in the brain (OR = 0.99, p = 0.99). Head injury was not a significant predictor of AD pathology after controlling for age (OR = 0.86, p = 0.89).

**Discussion and conclusion:** No association was seen between head injury and rate of disease progression in ALS. Head injury did not result in a specific neuropathologic phenotype in ALS. A subset of ALS autopsy cases, both with and without head injury, demonstrate that tau pathology can be described with chronic traumatic encephalopathy. These findings do not support a causal relationship between head injury and ALS.

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## C43 PREDICTING PROGNOSIS IN ALS: A SIMPLE ALGORITHM

ELAMIN M[1], BEDE P[1], MONTUSCHI A[3], CHIÒ A[3], HARDIMAN O[1,2]

[1]*Trinity Biomedical Sciences Institute, Dublin, Ireland,* [2]*Beaumont Hospital, Dublin, Ireland,* [3]*ALS Regional Expert Centre, University of Torino, Torino, Italy*

*Email address for correspondence: marwaelamin08@gmail.com*

*Keywords: prognosis, survival, progression*

**Background:** A validated, reliable and practical prognostic model for ALS patients is urgently needed to aid in the planning of care for individual patients, and to allow more efficient stratification procedures in clinical trials.

**Objectives:** We aimed to interrogate data pertaining to deeply phenotyped population-based samples of ALS patients, with the view to identifying a reliable prognostic algorithm using only clinical information that can be gathered the first time an ALS patient is evaluated.

**Methods:** The formulation of the prognostic index and internal validation was carried out using data generated as part of a large scale population-based study of cognitive function in Irish ALS patients. Recruitment for this study was reliant on the Irish ALS register.

External validation was carried out in a random sample of Italian ALS patients. This patient cohort was a random sub-cluster from a large-scale study where ALS patients resident in the provinces of Torino and Cuneo of Piemonte region, Italy, were identified through the Piemonte and Valle d'Aosta register for ALS and invited to participate.

Detailed clinical and neuropsychological data were available for both cohorts.

Significant predictors of survival time were identified in the Irish cohort using Kaplan-Meier methods and Cox proportional hazards. Internal validation of the model was carried out using boot-strapping techniques in 1000 random samples to obtain 95% confidence.

A prognostic index, generated by assigning weighted scores to each factor based on the hazard ratios suggested by the multivariate cox proportional model, was used to classify patients into prognostic risk subgroups. The utility of the risk group classification was tested in the Irish Cohort and (for external validation purposes) the Italian cohort.

**Results:** Data from 204 Irish ALS patients and 122 Italian patients was included. Mean patient age in the two cohorts was 61.5 and 65.5 years respectively with males representing 57.7% and 67.7% of the participants respectively.

On univariate analyses, significant predictors of survival time in the Irish population included (1) older age at symptom onset, $p = 0.024$; (2) Bulbar or respiratory (ie, non-spinal) onset of disease ($p = 0.006$); (2) rapid decline in ALSFR-R over time prior to time of evaluation ($p < 0.0001$); (3) and the presence of executive dysfunction, ($p < 0.0001$). Predictors whose survival effect persisted on multivariate analyses (with boot-strapping technique) were included in the prognostic and risk group classification (high, moderate and low risk groups).

In both Irish and Italian cohorts, allocated patient risk groups had a significant effect on observed median survival time with minimal overlap of the 95% confidence intervals (log rank test $p < 0.0001$ in both cases).

**Discussion and conclusion:** Our data suggest that a simple index using information that can be gathered on first clinical evaluation of ALS patients yields reliable information regarding individual patient prognosis.

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## C44 VALIDATION OF A SIMPLE SURVIVAL SCORE FOR PATIENTS WITH ALS (ALS-SS)

LUNETTA C, LIZIO A, MAESTRI E, SANSONE V

*Centro Clinico NEMO, Milan, Italy*

*Email address for correspondence: christian.lunetta@centroclinico nemo.it*

*Keywords: survival, score, clinical trials*

**Background:** A general concern in ALS is the huge variability of progression among patients, making it difficult to predict disease trend in individual cases. For therapeutic trials, we need to have a better understanding of the prognostic factors that may affect the disease course and limit interpretation of results during trials.

**Objective:** To identify prognostic factors for survival in a large population of patients with ALS followed by an tertiary care center; to create and validate a survival score for ALS.

**Methods:** The study included 298 patients, males (58%) and females (42%), with median disease duration of 15 months. Patients with tracheostomy were excluded. The following clinical and biochemical variables available at the time of first examination were recorded: family history; ethnicity; gender; age at onset; Riluzole use; site of onset; ALSFRS-R total score; % of Forced Vital Capacity (FVC); weight (kg); serum albumin (gr/L); aspartate aminotransferase or AST (U/L); serum chloride (mml/L), to assess their effect on survival. To determine which variables were independently correlated with survival we used univariate and multivariate Cox models.

**Results:** Using uninvariate Cox models we found that, at the time of the first examination family history, age, site of onset, weight, AST, serum chloride, serum albumin and ALSFRS-R total score were significantly associated to survival. According to the results of the multivariate analysis, family history, age, AST, and ALSFRS-R total score were included in the scoring system. Considering the survival probability, each factor was arbitrarily assigned a score ranging from 5 to 15 points (pts). These total scores, ALS-Survival Score (ALS-SS), represented the sum of these scores with values included from 20 to 60 pts. Two prognostic groups were formed with a significant difference for survival at Kaplan-Meyer's analysis ($p = 0.0012$). In addition, the ALS- SS was also validated using data from the Pooled Resource Open-Access ALS Clinical Trials Consortium (PRO-ACT) database and gave the same results ($p < 0.0001$).

**Discussion and conclusion:** ALS has a considerable variability in outcome and its prognostic factors are not satisfactorily defined (1–4). This simple survival score, obtained from independent prognostic factors influencing survival, appears valid and reproducible. Due to its feasibility it can be used to estimate the survival time of patients with ALS both routinely in the clinical context and in clinical trial studies.

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PRO-ACT database for the data used for the validation group. The authors report no conflicts of interest.

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# SESSION 7A GENETICS AND GENOMICS

## C45 FINDING ALS GENES BY MEANS OTHER THAN LINKAGE

HARDY J

*Reta Lila Weston Research Laboratories, Department of Molecular Neuroscience, UCL Institute of Neurology, London, UK*

*Email address for correspondence: j.hardy@ion.ucl.ac.uk*

*Keywords: genetic association, gene expression, bioinformatics*

After the identification of the SOD1 locus for ALS in the early 1990s, there was a long drought during which no new genes for ALS were discovered despite the fact that SOD1 mutations explained a only small proportion of the familiarity of the disease.

In the last 7 years there have been a wealth of new genetic discoveries and now there are many genes known which cause a pure ALS phenotype and many others which cause a phenotype that includes ALS and frontotemporal dementia. These findings now explain about half the familial clustering of the disorder. Additionally, the power of genetic technologies means it is likely that the other genetic loci will be discovered in a reasonable time frame.

In my talk, I will discuss how the loci for ALS seem to be mapping to specific pathways and this, together with expression networks should allow the identification of both other ALS loci and the more accurate delineation of the pathways to disease.

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## C46 A NOVEL LOCUS AT CHROMOSOME 1P ASSOCIATED WITH SURVIVAL IN PATIENTS WITH SPORADIC AMYOTROPHIC LATERAL SCLEROSIS IDENTIFIED THROUGH AN INTERNATIONAL GENOME WIDE META-ANALYSIS

FOGH I[1], LIN K[2], GELLERA C[3], TILOCA C[4], SHATUNOV A[1], RATTI A[4], D'ALFONSO S[5], DEL BO R[6], DOBSON RJ[7], CEREDA C[8], HARDIMAN O[11], LANDERS JE[12], VAN DAMME P[10], VELDINK JH[12], SILANI V[4], AL-CHALABI A[1], POWELL JF[2], CONSORTIUM A[12], CONSORTIUM SLAGEN[4]

[1]*Department of Clinical Neuroscience,* [2]*Department of Neuroscience Institute of Psychiatry, King's College London, London, UK,* [3]*Unit of Genetics of Neurodegenerative and Metabolic Diseases, Fondazione IRCCS Istituto Neurologico "Carlo Besta", Milano, Italy,* [4]*IRCCS Istituto Auxologico Italiano, Department of Neurology and Laboratory of Neuroscience, Milano, Italy,* [5]*Department of Health Sciences, Interdisciplinary Research Center of Autoimmune Diseases, 'A. Avogadro' University, Novara, Italy,* [6]*Neurologic Unit, IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy,* [7]*NIHR Biomedical Research Centre for Mental Health and Biomedical Research Unit for Dementia at South London and Maudsley NHS Foundation Trust, London, UK,* [8]*Laboratory of Experimental Neurobiology, IRCCS 'C. Mondino' National Institute of Neurology Foundation, Pavia, Italy,* [9]*Department of Neurology, University Hospital Leuven, and Laboratory for Neurobiology, Vesalius Research Center, VIB, University of Leuven, Leuven, Belgium,* [10]*Academic Neurology Unit, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland,* [11]*Department of Neurology, University of Massachusetts Medical School, Worcester MA, USA,* [12]*Department of Neurology, Rudolf Magnus Institute of Neuroscience, University Medical Centre Utrecht, Utrecht, The Netherlands*

*Email address for correspondence: isabella.fogh@kcl.ac.uk*

*Keywords: genome wide association study (GWAS), imputation, cox proportional hazards regression analysis*

**Background**: The prognosis of amyotrophic lateral sclerosis (ALS) can vary widely. So that, although the median survival of patients is around 36 months, many patients can survive for more than 10 years (1). This wide variation suggests that modifier genes may influence survival in ALS patients.

**Objective**: To address this hypothesis, we performed a large international genome wide association study (GWAS) of sporadic cases and analysed imputed data employing a Cox proportional hazards model to identify genes influencing survival.

**Methods**: We have undertaken a GWAS meta-analytical study of survival in patients with sporadic ALS, including 898 newly genotyped Italian case samples collected by SLAGEN (Italian Consortium for the Genetics of ALS) and case samples from Netherlands, USA, UK, Sweden, Belgium, France, Ireland and Italy collected by ALSGEN (the International Consortium on Amyotrophic Lateral Sclerosis Genetics). In total the international collection included seven independent GWAS studies with survival information available for 4,160 patients with genomic coverage extended by imputation analysis (1000 Genomes Project build 37/h19). Cox proportional hazards analysis was performed separately in each study using the ProbABEL package (http://www.genabel.org/) and results combined in a meta-analysis using METAL (http://www.sph.umich), weighting effect size estimates, or β-coefficients, using the inverse of the corresponding standard errors. The most associated variants will be validated in additional cohorts including a novel British cohort of 913 cases.

**Results**: We analysed 7,174,392 originally genotyped and imputed variants and identified a novel locus at chromosome 1p strongly associated with survival. Cox regression analyses were adjusted for population stratification by the specific principal components obtained from EIGENSTRAT analysis including: gender; age at onset; and site at onset, as covariates. Statistical genome-wide significance was reached by 25 common SNPs (MAF $> 0.24$) with $P$ values that ranged from $1.23 \times 10^{-9}$ to $4.75 \times 10^{-8}$. Further bioinformatic analysis of the most significant SNPs including eQTL analysis is now underway to allow prioritization for replication studies.

**Discussion and conclusion**: This is the largest genetic analysis of survival in ALS to date. We identified a locus strongly associated with survival providing evidence that sufficiently large sample sets with densely imputed SNP coverage can identify common variants associated with ALS phenotypes. This supports the use of survival analyses in ALS genetic studies, provides new insights into factors influencing the progression rate of sporadic ALS, and indicates new pathways that could be attractive drug targets.

**Acknowledgements**: This project is supported by funding from the Motor Neurone Disease Association UK. Sample selection and DNA preparation of data are described elsewhere (2).

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## C47 GENETIC DISEASE MODIFIERS IN INDIVIDUALS WITH C9ORF72 REPEAT EXPANSIONS

DICKSON D [1], RADEMAKERS R [1], VAN BLITTERSWIJK M[1], MULLEN B[1], WOJTAS A[1], HECKMAN M[2], DIEHL N[2], BAKER M[1], DEJESUS-HERNANDEZ M[1], BROWN P[1], MURRAY M[1], HSIUNG G[3], W[3], KARYDAS A[4], FINGER E[5], KERTESZ A[5], BIGIO E[6], WEINTRAUB S[6], MESULAM M[6], HATANPAA K[7], WHITE III C[7], NEUMANN M[8] STRONG M[9], BEACH T[10], WSZOLEK Z[11], LIPPA C[12], CASELLI R[13], PETRUCELLI L[1], JOSEPHS K[14], PARISI J [14], KNOPMAN D[14], PETERSEN R[14], MACKENZIE I[15], SEELEY W[4], GRINBERG L[4], MILLER B[4], BOYLAN K[11], GRAFF-RADFORD N[11], BOEVE B[14]

[1]*Mayo Clinic Department of Neuroscience,* [2]*Mayo Clinic, Section of Biostatistics, Jacksonville, FL, USA,* [3]*Division of Neurology, Vancouver, Canada,* [4]*University of California, Department of Neurology, San Francisco, CA, USA,* [5]*The University of Western Ontario, London, Canada,* [6]*Northwestern University Feinberg School of Medicine, Chicago, IL, USA,* [7]*University of Texas Southwestern Medical Center, Dallas, TX, USA,* [8]*University of Tubingen, Department of Neuropathology, Tubingen, Germany,* [9]*Robarts Research Institute, London, Canada,* [10]*Banner Sun Health Research Institute, Sun City, AZ, USA,* [11]*Mayo Clinic, Department of Neurology, Jacksonville, FL, USA,* [12]*Drexel University College of Medicine, Philadelphia, PA, USA,* [13]*Mayo Clinic, Department of Neurology, Phoenix, AZ, USA,* [14]*Mayo Clinic, Department of Neurology, Rochester, MN, USA,* [15]*Department of Pathology and Laboratory Medicine, Vancouver, Canada*

*Email address for correspondence: VanBlitterswijk.Marka@mayo.edu*

*Keywords: C9ORF72, disease modifier*

**Background:** The most frequent genetic cause of frontotemporal dementia (FTD) and motor neuron disease (MND) is a repeat expansion in the chromosome 9 open reading frame 72 (C9ORF72). Individuals with C9ORF72 repeat expansions can demonstrate substantial phenotypic heterogeneity, including variability in age at onset and survival after onset. To date, only a few disease modifiers have been reported, such as C9ORF72 expansion size and variants in transmembrane protein 106 B (TMEM106B).

**Objective:** To identify genetic disease modifiers that could explain the phenotypic heterogeneity observed in carriers of C9ORF72 repeat expansions.

**Methods:** A large cohort of 330 C9ORF72 expansion carriers and 374 controls was investigated. MassArray iPLEX and Taqman genotyping assays were used to examine variants previously implicated in FTD and/or MND; 36 variants were included in our analysis. Logistic regression models (disease risk), linear regression models (age at onset), and Cox proportional hazards regression models (survival after onset) were utilized to assess genetic associations. The predictive ability of significant associations was determined using R-squared (age at onset associations) and c-index (survival after onset associations) measurements.

**Results:** After adjustment for multiple testing, we discovered three variants significantly associated with age at onset in our overall cohort, including UBAP1 (rs7018487), PRNP (rs6052771) and MT-Ie (rs7403881). Additionally, we identified significant associations with survival after onset for six variants. Of those associations, one was present in our overall group, GRN (rs5848), three were observed in our FTD subgroup: MT-Ie (rs7403881); ELP3 (rs13268953); and the epsilon 4 allele (APOE), and two were seen in our MND subgroup: UNC13A (rs12608932) and ALAD (rs1800435). The associations identified through this study showed ample predictive ability; for instance, the three variants associated with age at onset explained more than 10% of the variability in age at onset.

**Discussion and conclusion:** Our study reveals eight novel disease modifiers that in part, elucidate the large phenotypic variability described in C9ORF72 expansion carriers. While these genetic variants have previously been implicated in FTD and/or MND, our study shows for the first time their modifying effect in the presence of a clear pathogenic mutation (ie, C9ORF72 repeat expansion). These novel disease modifiers also highlight the importance of protein degradation, antioxidant defence and RNA-processing pathways in the pathogenesis of C9ORF72-related diseases, and additionally, they are promising targets for the development of therapeutic strategies and prognostic tests.

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## C48 EXOME SEQUENCING IDENTIFIES MATRIN 3 AS A NEW ALS GENE

JOHNSON J[1], PIORO E[2], BOEHRINGER A[3], CHIA R[1], RESTAGNO G[4], SABATELLI M[5], BOWSER R[3], CHIÒ A[6], TRAYNOR BJ[1,7]

[1]*National Institute on Aging, Bethesda, MD, USA,* [2]*Cleveland Clinic, Cleveland, OH, USA,* [3]*Barrow Neurological Institute, Phoenix, AZ, USA,* [4]*A.S.O. O.I.R.M.-S. Anna, Turin, Italy,* [5]*Catholic University, Rome, Italy,* [6]*University of Turin, Turin, Italy,* [7]*Johns Hopkins Hospital, Baltimore, MD, USA*

*Email address for correspondence: traynorb@mail.nih.gov*

*Keywords: genomics, exome sequencing, matrin 3*

**Background:** Unravelling the genetic aetiology of ALS has provided fundamental insights into the cellular mechanisms underlying neuron degeneration, as well as facilitating disease modelling and the design and testing of targeted therapeutics. The pace of gene discovery has greatly accelerated, fuelled in large part by advances in sequencing and genotyping technology and we now know the genetic aetiology of two-thirds of familial cases and about 10% of sporadic ALS cases. Nevertheless, much remains to be discovered about the genetic architecture of ALS (1).

**Objectives:** To address this gap in our knowledge, we undertook an exome sequencing project to identify causative variants in familial ALS.

**Methods:** We used exome sequencing to identify shared, coding variants in the exome of affected individuals.

**Results:** Using exome sequencing, we identified a p.Phe115-Cys amino acid change in the Matrin 3 (MATR3) gene in a family with ALS and dementia (2). A p.Ser85Cys mutation in MATR3 has previously been described as a cause of distal asymmetrical myopathy with vocal cord paralysis in a large

family of European descent. Re-examination of affected members from this kindred led us to reclassify their condition as a slowly progressive form of ALS. Screening of MATR3 in additional ALS cases found two further mutations (p.Thr622Ala and p.Pro154Ser).

**Discussion and conclusion:** MATR3 is an RNA/DNA binding nuclear protein, thought to interact with TDP-43, which itself is involved in ALS pathogenesis. We have also observed a novel MATR3 pathology in the spinal cords and brains of ALS cases with and without MATR3 mutations (2). This data provides additional evidence supporting the role of aberrant RNA processing in motor neuron degeneration.

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# SESSION 7B END OF LIFE DECISIONS

## C49/C50/C51 ASSISTED DYING AND ALS/MND

BORASIO GD[1], FINLAY IG[2], VAN DEN BERG LH[3]

[1]*CHUV, University of Lausanne, Lausanne, Switzerland,* [2]*Cardiff University, Cardiff, UK,* [3]*University Medical Center, Utrecht, The Netherlands*

*Email address for correspondence: Gian.Borasio@chuv.ch*

*Keywords: assisted dying, euthanasia, assisted suicide*

ALS patients have featured prominently in several high-profile cases involving requests for assisted dying. Diane Pretty, a British ALS patient, petitioned the European Court of Human Rights to allow her husband to assist her suicide (a crime according to British law), on account of the "horrible death by choking" that was allegedly awaiting her. Her request was denied, and she died peacefully in a hospice.

The relentless progression of ALS, coupled with intact cognitive functions in the majority of patients, provides the backdrop for end-of-life scenarios which are perceived by some patients as potentially causing them unbearable suffering (1).

In those jurisdictions that allow 'assisted dying', the proportion of ALS patients that resort to this option is among the highest of all diseases reported (2). Wishes for hastened death have been shown to be frequent in ALS patients (3). On the other hand, palliative care in ALS has the best evidence base of all neurodegenerative disorders so far (4), with patients often fearful of situations that are amenable to timely intervention.

Several countries and states are currently debating whether to introduce legislation allowing 'assisted dying' (France, England) or have recently done so (Québec). ALS cases were often showcased during the political discussions. But is 'assisted dying' really the solution to difficult end of life situations in ALS?

In this session, we would like to present and discuss different viewpoints on this controversial issue. Available data from the Benelux countries, which allow active euthanasia, will be compared to data from regions that only allow assisted suicide (Oregon and Switzerland). The palliative care viewpoint will be presented in detail.

We hope to foster a discussion that will look at end of life decisions in ALS without prejudice. We feel that the ethical principles of patient autonomy and benevolence are not mutually exclusive but complementary, and that respect for the patient's choices as well as for the health care professionals' ethical and evidence-based perspective are fundamental components of good end of life care.

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# SESSION 7C BIOMARKERS (I)

## C52 NEUROFILAMENT LIGHT CHAIN IN BLOOD IS A PROGNOSTIC, AND A POTENTIAL PHARMACODYNAMIC BIOMARKER FOR AMYOTROPHIC LATERAL SCLEROSIS

LU C-H[1,2], MACDONALD-WALLIS C[3], GRAY E[4], PEARCE N[5], PETZOLD A[6], NORGREN N[7], GIOVANNONI G[1], FRATTA P[8], SIDLE K[9], FISH M[10], ORRELL R[11], HOWARD R[12], TALBOT K[4], GREENSMITH L[2,13], KUHLE J[1,14], TURNER M[4], MALASPINA A[1,15]

[1]*Centre for Neuroscience and Trauma, Blizard Institute, Queen Mary University of London, London, UK,* [2]*Sobell Department of Motor Neuroscience and Movement Disorders, UCL Institute of Neurology, London, UK,* [3]*MRC Integrative Epidemiology Unit, University of Bristol, Bristol, UK,* [4]*Nuffield Department of Clinical Neuroscience, University of Oxford, Oxford, UK,* [5]*Department of Medical Statistics, London School of Hygiene and Tropical Medicine, London, UK,* [6]*Department of Neuroinflammation, UCL Institute of Neurology, London, UK,* [7]*UmanDiagnostics, Umeå, Sweden,* [8]*Department of Neurodegenerative Disease,* [9]*Department of Molecular Neuroscience, UCL Institute of Neurology, London, UK,* [10]*Medicine Clinical Trial Unit, Musgrove Park Hospital, Taunton, UK,* [11]*Department of Clinical Neuroscience, UCL Institute of Neurology, London, UK,* [12]*National Hospital for Neurology and Neurosurgery, London, UK,* [13]*MRC Centre for Neuromuscular Diseases, UCL Institute of Neurology, London, UK,* [14]*Neurology, University Hospital Basel, Basel, Switzerland,* [15]*North-East London and Essex MND Care and Research Centre, London, UK*

*Email address for correspondence: c.lu@qmul.ac.uk*

*Keywords: biomarkers, longitudinal analysis, prognostic and treatment response*

**Background:** Disease progression as well as mortality is a critical therapeutic outcome measure in amyotrophic lateral sclerosis (ALS), a clinically heterogeneous and fatal neurodegenerative disorder. Neurofilament light chain (NfL), as the main break down products of neurodegeneration, have been variably elevated in small cross-sectional studies of blood and cerebrospinal fluid (CSF) in ALS (1–3).

**Objectives:** To investigate whether blood NfL level is a disease progression and prognostic biomarker in ALS.

**Methods:** Using an electrochemiluminescence ELISA assay, NfL levels were measured in plasma, serum and CSF samples from two large cohorts of ALS patients and healthy controls, recruited independently in London (ALS/Control: n = 103/42) and Oxford (ALS/Control: n = 64/36). NfL levels in patients were measured at regular intervals for up to two years. Change in the revised ALS functional rating scale revised (ALSFRS-R) over time was used to evaluate the rate of disease progression. A multilevel random intercept model with a linear slope was used to examine NfL longitudinal trajectories in three ALS progression subgroups: slow, intermediate and fast progressors. Survival analysis was undertaken using Kaplan-Meier analysis and a Cox proportional hazards modelling.

**Results:** CSF, serum and plasma NfL discriminated ALS patients from healthy controls with high sensitivity (97%, 89%, 90% respectively) and specificity (95%, 75%, 71% respectively). CSF NfL levels were highly correlated with matched serum NfL levels ($r = 0.781$, $p < 0.0001$). Blood NfL levels at baseline were approximately four times as high in ALS patients compared to controls in both London and Oxford cohorts and were strongly correlated with disease progression rate at baseline ($r = 0.468$ and 0.512 in London and Oxford cohort, respectively; $p < 0.0001$). Both cohorts displayed a steady but distinct blood NfL expression in ALS patients in the follow-up period. Blood NfL levels at recruitment and other clinical covariates were strong independent predictors of survival. The highest tertile of blood NfL at baseline (compared with the lowest tertile) had a mortality hazard ratio (HR) of 3.82 (95% CI 1.98–7.39, $p < 0.001$).

**Discussion and conclusion:** NfL in blood is a readily available prognostic biomarker in ALS. This is an important advance both for individualised care planning, and for wider stratification in the improved evaluation of therapeutic responses. The steady levels of NfL longitudinally offer potential as a pharmacodynamic biomarker in future therapeutic trials.

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## C53 CSF NEUROFILAMENT LIGHT CHAIN CONCENTRATION REFLECTS CORTICOSPINAL TRACT MICROSTRUCTURE IN ALS

MENKE R[1], GRAY E[1], LU C-H[2], TALBOT K[1], MALASPINA A[2], KUHLE J[3], TURNER M[1]

[1]*Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, UK,* [2]*Centre for Neuroscience and Trauma, Blizard Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, UK,* [3]*Neurology, University Hospital Basel, Basel, Switzerland*

*Email address for correspondence: ricarda.menke@ndcn.ox.ac.uk*

*Keywords: diffusion tensor imaging, neurofilament light chain (Nfl), biomarkers*

**Background:** Neurofilament light chain protein (NfL) in cerebrospinal fluid (CSF) is thought to reflect axonal damage, and is a leading prognostic neurochemical biomarker for ALS. Magnetic resonance diffusion tensor imaging (DTI) measures, such as decreased fractional anisotropy (FA) and increased radial diffusivity (RD) have been shown to be sensitive to microstuctural white matter alterations. A core DTI white matter signature involving the corticospinal tracts (CSTs) and motor callosal fibres has been consistently identified in ALS patients at the group level.

**Objectives:** To investigate the relationship between CSF NfL levels and DTI measures of white matter microstructural integrity in ALS patients and healthy controls, with clinical correlations undertaken in the patient group.

**Methods:** DTI data acquired at 3 Tesla and matched CSF NfL concentrations measured using an electrochemilumines-

cence-based sandwich immunoassay were obtained from members a cohort of ALS patients (n = 25) and healthy controls (n = 17) as part of The Oxford Study for Biomarkers in Motor Neuron Disease (BioMOx). For both groups, correlations (corrected for age) between CSF NfL concentrations and DTI measures in three different white matter tracts (left and right CST, corpus callosum (CC), and left and right superior longitudinal fasciculi (SLF)) were performed. NfL concentrations were separately correlated with age, upper motor neuron (UMN) score, and progression rate (rate of decline in ALSFRS-R) in the ALS group.

**Results:** Mean CSF NfL levels were significantly higher in patients (7118 ± 4879 pg/ml) than controls (663 ± 464 pg/ml; $p < 0.0001$). In controls, NfL concentration was positively correlated with age ($r = 0.742$, $p = 0.001$). In patients, NfL levels correlated positively with UMN score ($r = 0.461$, $p = 0.020$), and progression rate ($r = 0.902$, $p < 0.0001$). DTI analysis revealed significant ($p < 0.05$) negative correlation between NfL measures and FA (co-localized with positive RD correlation) in both CSTs. In controls, only a positive correlation between CSF NfL concentration and RD for a small region in the left SLF was observed.

**Discussion and conclusion:** Elevated CSF NfL concentrations in ALS are related to white matter microstructure damage within the CSTs as measured with DTI. The combination of both a neurochemical and neuroimaging biomarker may now be applicable at the individual subject level in ALS, and prospective studies in relevant patient groups are underway.

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## C54 MULTI-CENTER VALIDATION OF A DIAGNOSTIC ASSAY FOR ALS

BOWSER R[1,2], AN J[1], KOVALIK T[1], SHAW G[2], JEROMIN A[2]

[1]*Barrow Neurological Institute, Phoenix, AZ, USA,* [2]*Iron Horse Diagnostics, Inc., Phoenix, AZ, USA*

*Email address for correspondence: Robert.Bowser@Dignity Health.org*

*Keywords: biomarkers, neurofilament, diagnostic test*

**Background:** There is a critical need for biomarkers that can accurately predict ALS and biomarkers that are prognostic indicators of disease. Biofluids (blood, cerebrospinal fluid (CSF), urine) have been used to discover protein or metabolic biomarkers of disease. However these candidate biomarkers must be validated in large, prospective clinical research studies using well-characterized assays. We reported a CSF based biomarker for ALS that combines phosphorylated neurofilament heavy chain (pNFH) and complement C3 that could accurately predict ALS (1). We, and others have also reported increased levels of pNFH in the blood of patients with ALS (2). To further evaluate the diagnostic utility of these assays, we must fully characterize the immunoassays and perform a multicentre prospective study to test the overall accuracy of these tests at predicting ALS.

**Objective:** The goal was to perform a blinded, multicentre validation of a diagnostic assay for ALS. CSF and blood plasma was prospectively collected from 214 subjects at neuromuscular clinics at 30 medical centres in the USA using standard operating procedures and shipped to the NEALS biorepository.

**Methods:** Coded samples were shipped from the central biorepository to the Bowser laboratory for analysis. Meso Scale Discovery (MSD) immunoassays were first optimized for each biofluid following which, the levels of pNFH in the blood and CSF, and complement C3 in the CSF were quantified. Diagnostic predictions were made using a pNH/complement C3 ratio in the CSF and pNFH levels in the plasma, using previously published cut-off values for the CSF and plasma assays (1, 2). The central biorepository broke the code and determined the accuracy of the diagnostic predictions.

**Results:** Using the pNFH/c3 ratio in the CSF, we were 93% accurate at predicting ALS (sensitivity = 92.5%, specificity = 93.2%). With the plasma based pNFH assay, we predicted ALS with 70% accuracy (sensitivity = 65%, specificity = 75%).

**Discussion and conclusion:** We performed a prospective validation of a diagnostic test for ALS using samples collected from 225 subjects at 30 medical centres. The CSF based assay was 93% accurate at predicting ALS. Our results indicate that these CSF and blood based assays may assist clinicians in making an earlier and accurate diagnosis of ALS. Earlier diagnosis will enable enrolment of patients into clinical trials at an earlier stage of disease. The immunoassays for pNFH and complement c3 have completed assay analytical validation at Iron Horse Diagnostics, Inc. A final prospective clinical qualification study within a certified central laboratory is currently underway using 4 sites in the US and 2 sites in Europe.

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## C55 EVALUATION OF OXIDATIVE STRESS AND OTHER BIOMARKERS AT THE BASELINE OF A LARGE ALS COHORT STUDY (ALS COSMOS)

MITSUMOTO H[1], SANTELLA R[2], CREMERS S[3], FURLONG C[4], VOTTO T[5], HUPF J[1], SINGLETON J[1], GURVICH I[2], FACTOR-LITVAK P[5], ALS COSMOS STUDY GROUP[1]

[1]*Eleanor and Lou Gehrig MDA/ALS Research Center, Columbia University Medical Center, New York, New York, USA,* [2]*Department of Environmental Health Sciences, Columbia University, New York,* [3]*Irving Institute for Clinical and Translational Research, Columbia University Medical Center, New York, New York, USA,* [4]*Departments of Genome Sciences and Medicine, University of Washington, Seattle, Washington, USA,* [5]*Department of Epidemiology, Mailman School of Public Health, Columbia University, New York*

*Email address for correspondence: hm264@cumc.columbia.edu*

*Keywords: biomarkers, oxidative stress, disease status*

**Background:** Clinimetrics is the only validated method to evaluate functional status or disease progression in ALS. Identifying reliable biomarkers is essential for objectively assessing ALS function and progression.

**Objective:** To evaluate multiple biomarkers in patients with ALS at the start of a prospective, large cohort, longitudinal multicenter studies (ALS COSMOS) to investigate associations between these biomarkers and ALS function.

**Methods:** Data and biosamples were collected at the time of first clinical evaluation (1). ALSFRS-R and percentage forced vital capacity (% FVC) were used to assess ALS clinical status. We measured two urinary biomarkers of oxidative stress in overnight-fasting, spot first morning voids: isoprostane, a product of lipid peroxidation, and 8-oxo-deoxyguanosine (8-oxo-dG), a product of DNA oxidation; both were adjusted for urinary concentration using specific gravity. Plasma creatinine, extensive lipid profile, and paraoxonase 1 (PON1) were also determined. Associations were evaluated using linear regression, controlling for patient reported duration of symptoms and potential confounders.

**Results:** 355 patients were enrolled in ALS-COSMOS, of whom 324 had ALS-FRS data, 325 had FVC data, 338 had urine biomarker data and 337 had plasma creatinine data. Mean (+ standard deviation (SD)) values of urinary isoprostane and 8-oxo-dG, both adjusted for specific gravity were 1.60 (0.98) and 17.1 (13.8), respectively. Mean plasma creatinine was 0.80 (0.20). After control for duration of symptoms, age, sex, race, ethnicity, and BMI, each 0.1 unit increase in serum creatinine was associated with a 0.91 unit increase in ALSFRS-R ($p < 0.0001$) and with a 2.38% increase in % FVC ($p = 0.0006$). Similarly, each unit increase in 8-oxodG was associated with a 0.10 point decrease in ALSFRS-R ($p = 0.0080$) and with a 0.25% decrease in % FVC ($p = 0.0751$). Isoprostane showed a trend in the direction similar to 8-oxodG.

**Discussion and conclusion:** In this population, we found associations between plasma creatinine and ALSFRS-R and respiratory function such that higher serum creatinine was associated with better function. These results are similar to others reported in the literature. We also found associations between two biomarkers of oxidative stress and decreases in ALS-FRS and % FVC. No associations were found for PON1 activity in this baseline data, after stratifying by sex and PON1 genotype.

**Acknowledgements:** NIEHS (R01ES160348) and MDA Wings Over Wall Street.

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## C56 THE ROLE OF ALBUMIN AND CREATININE IN A POPULATION-BASED COHORT OF ALS PATIENTS

CALVO A[1,2], BOVIO G[3], CANOSA A[1,4], BERTUZZO D[1], GALMOZZI F[1], CUGNASCO P[1], CLERICO M[5], DE MERCANTI S[5], BERSANO E[6], CAMMAROSANO S[1], ILARDI A[1], MANERA U[1], MOGLIA C[1], MARINOU K[7], BOTTACCHI E[8], PISANO F[9], CANTELLO R[6], MAZZINI L[6], MORA G[9], CHIÒ A[1,2]

[1]*ALS centre, Department of Neuroscience "Rita Levi Montalcini", University of Torino, Torino, Italy,* [2]*Azienda Ospedaliero Universitaria Città della Salute e della Scienza, Torino, Italy,* [3]*Salvatore Maugeri Foundation, IRCSS, Scientific Institute of Pavia, Pavia, Italy,* [4]*Department o Neuroscience, Opthalmology, Genetics, Rehabilitation and Child Health, University of Genova, Genova, Italy,* [5]*Department of Biological and Clinical Science, University of Turin, and Azienda Ospedaliero Universitaria San Luigi Gonzaga, Orbassano, Italy,* [6]*ALS Center, Department of Neurology, Azienda Ospedaliero Universitaria Maggiore di Novara, Novara, Italy,* [7]*Salvatore Maugeri Foundation, IRCSS, Scientific Institute of Milano, Milano, Italy,* [8]*Department of Neurology, Azienda Ospedaliera Regionale di Aosta, Azienda USL Valle d'Aosta, Aosta, Italy,* [9]*ore Maugeri Foundation, IRCSS, Scientific Institute of Veruno, Veruno, Italy*

*Email address for correspondence: andreacalvo@hotmail.com*

*Keywords: prognosis, albumin, creatinine*

**Background:** There is an urgent need to identify reliable biomarkers of Amyotrophic Lateral Sclerosis (ALS) progression both for clinical practice and pharmacological trials.

**Objective:** To correlate several haematological markers evaluated at diagnosis with ALS outcome in a population-based series of patients ('discovery' cohort). To replicate the findings in an independent 'validation cohort' from an ALS tertiary center.

**Methods:** The discovery cohort included ALS patients from the Piemonte /Valle d'Aosta Register for ALS, in the 2007–2011 period. The validation cohort comprised 122 ALS patients at different stages of disease consecutively seen at an ALS tertiary center between 2007 and 2009. The following haematological factors were investigated and correlated to survival: total leukocytes; neutrophils; lymphocytes; monocytes; glucose; creatinine; uric acid; albumin; bilirubin; total cholesterol; triglycerides; high density lipoproteins; low density lipoproteins; creatine kinase; thyroid stimulating hormone; and erythrocyte sedimentation rate (ESR); all analyses were performed separately for gender. The patients in the validation cohort also underwent bioelectrical impedance analysis for the calculation of fat-free mass (FFM).

**Results:** Of the 712 incident patients in the examined period in Piemonte/Valle d'Aosta, 638 (89.6%) were included in the study. Only serum albumin, serum creatinine and lymphocyte count were significantly related to ALS outcome in both genders, with a dose-response effect (better survival with increasing levels). These findings were confirmed in the validation cohort. Multivariable analysis showed that serum albumin and creatinine were independent predictors of survival in both genders; no other hematological factor was retained in the model. In ALS patients, serum albumin was correlated with markers of inflammatory state, while serum creatinine was correlated with FFM, which is a marker of muscle mass.

**Discussion and conclusion:** In ALS, serum albumin and creatinine are independent markers of outcome in both genders. Creatinine reflects the muscle waste whereas albumin is connected with inflammatory state. Both creatinine and albumin are reliable and cheap markers of the severity of clinical status in ALS patients that could be used in defining their prognosis at time of diagnosis.

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# SESSION 8A MURINE MODELS

## C57 DEVELOPMENT OF MOUSE MODEL FOR A NEWLY DISCOVERED MUTANT PROFILIN1 IN FALS PATIENTS

KIAEI M, YADAV S

*University of Arkansas for Medical Sciences, Little Rock, AR, USA*

*Email address for correspondence: mkiaei@uams.edu*

*Keywords: profilin1, actin polymerization, transgenic mouse model*

**Background:** Recently, five mutations in profilin1 (PFN1) gene (ALS18) were linked to a subpopulation of fALS patients that had none of the previously known mutated genes in fALS (1). PFN1 is a ubiquitously expressed small actin-binding protein essential for the regulation of filamentous F-actin formation from monomeric G-actin. Most of the PFN1 mutations identified in ALS patients are situated near the protein surface where PFN1 interacts with G-actin, resulting in disruption of actin polymerization, likely inhibiting axon and dendrite outgrowth. Whether profilin1 mutations in this group of ALS patients is a cause of ALS, remain unknown. Identification of PFN1 mutation in human ALS patients with approximately 10 years earlier on average age of onset than other ALS patents and common clinical limb onset makes a strong case for its involvement, but doesn't automatically confer that it is the cause.

**Objectives:** To address the cause and effect, and mechanism of profilin1 neurotoxicity, we developed transgenic mice that overexpress human profilin1 mutation and examined the animals for ALS-like phenotype to investigate the mechanism(s) of mutant PFN1 neurotoxicity.

**Methods:** Transgenic mice were developed using standard methods and were monitored for general wellbeing; behaviour; weight; motor performance and survival length using standard techniques.

**Results:** We have successfully developed transgenic mice overexpressing mutant human PFN1. Our profilin1 transgenic mice are viable, appear normal at birth and remain healthy enough to breed and generate viable offspring. Mutant PFN1 mice develop ALS-like phenotypes such as hindlimb fine tremor and clasping; gait abnormality leading to low body profile; reduced stride length; gradual weakness and atrophy in muscle of limbs; kyphosis; significant weight loss toward later part of the disease; and show a significantly reduced lifespan.

**Discussion and conclusion:** We have developed a new mouse model overexpressing a novel human gene with mutation found in fALS called ALS18. Overexpression of mutant human PFN1 in our mice resulted in the development of ALS-like phenotypes. To our knowledge this model is the first to be produced and develop symptoms and signs that resembles ALS. This model potentially can be used to investigate mutant profilin1 neurotoxicity in motor neurons and how it causes ALS. This model is expected to be useful for testing therapeutic strategies for development of therapy for ALS.

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## C58 RNA PROCESSING ALTERATIONS FROM ALS-LINKED MUTATIONS IN FUS/TLS

DA CRUZ S, PARONE PA, MCALONIS-DOWNES M, VETTO AP, BUI AT, JAMBEAU M, SUN Y, LAGIER-TOURENNE C, CLEVEL DW

*University of California San Diego, Ludwig Institute for Cancer Research, La Jolla, CA, USA*

*Email address for correspondence: sdacruz@ucsd.edu*

*Keywords: FUS/TLS, TDP-43, RNA metabolism*

**Background:** RNA binding proteins have emerged as central players in the mechanisms of neurotoxicity underlying many of the most prominent neurodegenerative diseases. In particular, mutations in two prototypical RNA binding proteins: TAR DNA-binding protein (TDP-43) and Fused in sarcoma/Translocated in liposarcoma (FUS/TLS) have been shown to cause ALS and FTLD. Both proteins have also been found to form pathological inclusions in several neurodegenerative conditions. Despite this, the physiological and pathological functions of TDP-43 and FUS/TLS within the central nervous system are poorly understood, and it is not known whether the mechanisms underlying neurotoxicity are caused by a gain of toxic property and/or a loss of function via their sequestration into aggregates.

**Objectives:** To determine how ALS-linked mutations in the FUS/TLS gene cause neurotoxicity and identify new targets for therapy development, we have combined the use of newly generated mouse models for FUS/TLS mediated disease and high-throughput sequencing methodologies to elucidate disease specific-RNA processing alterations.

**Results:** To identify functional alterations caused by ALS-linked FUS/TLS mutations without confounding the activity of endogenous FUS/TLS, we generated transgenic mice in which wild-type or ALS-linked mutants of human FUS/TLS replaced endogenous FUS/TLS (following disruption of both endogenous mouse FUS/TLS alleles and integration of the human FUS/TLS gene). We found: (1) the expression level and subcellular localization of human FUS/TLS mirrors that of mouse FUS/TLS in normal mice; (2) human wild type and mutant FUS/TLS both fully rescue the early postnatal lethality that would result from the lack of endogenous FUS/TLS expression; (3) the mice expressing ALS-linked mutants of human FUS/TLS develop adult onset progressive motor and cognitive deficits recapitulating aspects of ALS and FTLD diseases. RNA-seq and RASL-seq methodologies have been used to determine changes in RNA expression levels and splicing profiles associated with age-dependent disease caused by mutant FUS/TLS.

**Discussion and conclusion:** Determination of RNA signatures associated with age-dependent progressive neurodegen-

eration caused by ALS-linked mutants of FUS/TLS, identifies mutant-dependent disease mechanisms underlying neurotoxicity and provides the basis for developing novel therapeutic targets.

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## C59 ESTABLISHING A NOVEL ALS KNOCK-IN MOUSE MODEL WITH THE ALS 8 MUTATION

PERRINE G, LESLEY S, LARROQUETTE F, BAI L, TSUDA H

*Montreal Neurological Institute, McGill University, Montreal, Canada*

*Email address for correspondence: hiroshi.tsuda@mcgill.ca*

*Keywords: VAPB, ALS8, transgenic mice*

**Background:** To identify core pathological defects in ALS, we have been investigating molecular pathways associated with Vamp associated protein (Vap) B (1, 2), in which a missense (P56S) mutation causes a familial form of ALS, ALS 8 (3). Although SOD1 (ALS 1) and TDP-43 (ALS 10) transgenic mice overexpressing the mutant protein with both neuron-specific and ubiquitous promoters have provided some insight into the toxic properties of the mutant proteins, their role in pathogenesis remains unclear. In theory, expressing the mutant proteins in the correct temporal and spatial expression patterns will give us a better understanding of the mechanisms of cell-specific vulnerability and effects of the pathological ALS mutations. Therefore, it is essential to create animals expressing the ALS mutant protein at physiological levels in the appropriate tissues to analyze the resulting cellular pathological phenotypes.

**Methods:** To determine the core cellular biological defects of ALS using animal models we have generated *vapb* knock-in mice carrying the ALS 8 mutation and analysed the resulting cellular pathological phenotypes. To determine the link between Vap and other familial and sporadic form of ALS, we have examined if ALS 8 Vap leads to key pathological features implicated in ALS.

**Results:** We found that ALS 8 knock-in mice recapitulate many of the characteristic features of the disease; specifically ALS 8 knock-in mice show progressive defects in motor behaviours. Interestingly, the mice demonstrate accumulation of ubiquitinated proteins in the motor neurons in an age dependent manner similar to that observed in ALS patients. More importantly, TDP-43 (ALS 10) and FUS (ALS 6) proteins are mislocalized from the nucleus, where it is normally concentrated, to the cytoplasm. An identical cytoplasmic redistribution of TDP43 and FUS are characteristic of degenerating neurons from patients with ALS, suggesting that ALS8 mutant Vap causes defects in proper localization of TDP-43 and FUS resulting in the pathology of ALS.

**Discussion and conclusion:** The ALS 8 Vap knock-in mice will enable us to better understand the mechanisms by which the disease arises. Significantly, sporadic ALS patients have been shown to exhibit decreased levels of Vap in their spinal cords and cerebrospinal fluid, suggesting that Vap might also contribute to the pathogenesis of sporadic ALS. This study will have a transforming impact on our understanding of ALS pathogenesis and will provide clues for developing strategies to delay the course of the disease.

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## C60 PHYSIOLOGICAL LEVELS OF GENE EXPRESSION IN A BAC MODEL OF TDP-43-ASSOCIATED ALS LEAD TO AGE DEPENDENT MOTOR DEFECTS AND CYTOPLASMIC REDISTRIBUTION OF TDP-43

GORDON D, MUTIHAC R[1], FARRIMOND L[1], CHEUNG N[1], ALEGRE-ABARRATEGUI J[2], DAVIES B[3], ANSORGE O[4], WADE-MARTINS R[2], TALBOT K[1]

[1]*Nuffield Department of Clinical Neurosciences,* [2]*Department of Physiology, Anatomy and Genetics,* [3]*Wellcome Trust Centre for Human Genetics,* [4]*Department of Neuropathology, University of Oxford, Oxford, UK*

*Email address for correspondence: David.Gordon@ndcn.ox.ac.uk*

*Keywords: TDP-43, bacterial artificial chromosome, transgenic mouse model*

**Background:** TAR DNA-binding protein 43 (TDP-43) is the neuropathological hallmark protein of most cases of ALS. In affected motor neurons (MNs), TDP-43 becomes characteristically depleted from the nucleus and mislocalised to the cytoplasm where it forms the major protein component of insoluble, ubiquitinated inclusions.

**Objective:** Current mouse models of ALS are of uncertain relevance as they may reflect toxicity from TDP-43 overexpression. We have used site-specific integration of a BAC with wild-type (WT) or M337V TDP-43 to produce a model with physiological levels of human TDP-43 expression

**Methods:** Bacterial artificial chromosome (BAC) vectors containing the full length human genomic locus of the WT or M337V mutation, with a Ypet tag, were targeted to the ROSA26 locus in embryonic stem cells (ESC) by PhiC31 integrase mediated cassette exchange. Chimeric mice were generated by blastocyst injection of recombinant ESCs, which were subsequently crossed with C57BL/6J female mice to generate two isogenic human TDP-43 transgenic lines, differing only by the presence or absence of the M337V mutation.

**Results:** Human TDP-43 is expressed at physiological levels in TDP-43-BAC mouse CNS. Compared to controls, preclinical and clinical mutant mice display significantly elevated levels of TDP-43 protein species in the insoluble protein fractions from brain and spinal cord, as measured by immunoblotting. Primary MNs derived from E13.5 embryonic mutant mice recapitulate the characteristic cytoplasmic mislocalisation of TDP-43 under basal culture conditions. In response to increased oxidative stress (60 min), the number of mutant-derived MNs containing stress granules is significantly reduced compared to WT and non-transgenic (NTg) controls, with a concomitant reduction in co-localisation between human TDP-43 and stress granule markers in the cytoplasm. Mutant-derived MNs also show significantly reduced endoplasmic reticulum $Ca^{2+}$ stores. Longitudinal analysis of the CNS identifies nuclear clearing of TDP-43 from MNs in the ventral horn of mutant mouse spinal cord from 9 months of age, as

well as the presence of TDP-43-positive aggregates in the cytoplasm. p62 and phosphorylated TDP-43-positive aggregates are also observed in the spinal cord from 9 months of age. Both male and female homozygous mutants develop age-dependent, progressive motor deficits from 6–9 months of age in gait, motor function (accelerating rotarod) and grip strength.

**Discussion and conclusion:** Physiological levels of expression of mutant human TDP-43 in BAC transgenic mice, overcome the confounding effects of protein overexpression seen in other models and result in typical ALS pathology. In combination with ongoing longitudinal analysis (NMJ pathology, protein/RNA expression and RNAseq), timed to compare pre-clinical with various stages of clinical mice, this model will be a valuable tool to address the fundamental role of TDP-43 mutation in ALS and as an aid to pre-clinical testing of drugs with therapeutic potential.

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## C61 TRANSPLANT OF LIGHT-SENSITIVE STEM CELL-DERIVED MOTOR NEURONS TO ARTIFICIALLY RESTORE MUSCLE FUNCTION IN THE SOD1$^{G93A}$ MOUSE MODEL OF ALS/MND

BRYSON JB[1], MACHADO CB[2], LIEBERAM I[2], GREENSMITH L[1]

[1]*UCL Institute of Neurology, London, UK,* [2]*MRC Centre for Developmental Neurobiology, King's College London, London, UK*

*Email address for correspondence: barney.bryson@ucl.ac.uk*

*Keywords: stem cell-derived motor neuron, muscle reinnervation, optogenetics*

**Background:** In ALS, the loss of motor neurons prevents transmission of motor signals from the CNS to skeletal muscles, resulting in paralysis. We have taken a novel approach to restore function to paralysed muscles, which involves a synthesis of stem cell-derived neuronal replacement and optogenetics (1). We generated murine embryonic stem cell-derived motor neurons (ESC-MNs), modified to express channelrhodopsin-2 (ChR2) and glial derived neurotrophic factor (GDNF) to enable optogenetic control of their neural activity and to promote their survival, respectively. Following sciatic nerve ligation in wild type mice, which results in muscle denervation, we transplanted the ESC-MNs into specific branches of the sciatic nerve. These transplanted ESC-MNs not only successfully reinnervated distal muscle targets but, importantly, they were able to induce controllable muscle contraction *in vivo* when optically stimulated using 470nm light.

**Objectives:** We aim to establish whether these customized ESC-MNs can be successfully transplanted into peripheral nerves of SOD1$^{G93A}$ mice and maintain long-term innervation and optogenetic control of target muscles, as the next step to establishing the translational potential of this approach for the treatment of ALS patients.

**Methods:** ChR2$^{+}$Gdnf$^{+}$ ESC-MNs were transplanted into injured and uninjured branches of the sciatic nerve in SOD1$^{G93A}$ mice at pre- and post-symptomatic stages of disease. Mice were assessed using the following criteria: a) ESC-MN survival in a toxic environment; b) innervation of target muscles; c) induction of muscle contraction by optical stimulation.

**Results:** Our data indicates that these ESC-MNs can survive within the peripheral nerve environment of SOD1$^{G93A}$ mice until late-stage disease, even when transplanted after symptom onset. Moreover, these ESC-MNs maintain extensive axonal projections to distal muscle targets, at least up until 105 days (late-stage disease).

**Conclusions:** The results of this study advance the translational potential of this novel strategy as a means to restore function to paralyzed muscles in ALS patients.

**Acknowledgements:** We are grateful to the Motor Neurone Disease Association (UK) and Thierry Latran Foundation for supporting this study.

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# SESSION 8B RESPIRATORY MANAGEMENT

## C62 SCREENING FOR RESPIRATORY FAILURE IN ALS USING CLINICAL QUESTIONING, RESPIRATORY FUNCTION TESTS AND TRANSCUTANEOUS CARBON DIOXIDE: WHICH IS THE BETTER TOOL?

RAFIQ M, PROCTOR A, MCDERMOTT CJ, SHAW PJ

*SITraN, University of Sheffield, Sheffield, UK*

*Email address for correspondence: m.k.rafiq@sheffield.ac.uk*

*Keywords: respiratory failure, respiratory function tests, transcutaneous carbon dioxide*

**Background:** Screening patients regularly for evidence of respiratory failure is an important facet in the management of ALS. Standard practice is to screen patients for symptoms of respiratory failure and supplement this with one or more respiratory function tests. Forced vital capacity (FVC) is a widely used respiratory function test. An FVC of 50% predicts day time hypercapnia with a sensitivity of 53% and specificity of 89% (1). $PCO_2$ can be measured transcutaneously using TOSCA *500* (Linde Medical Sensors) (2).

**Objectives:** To evaluate the relative value of symptom history (using a structured questionnaire), respiratory function tests and day time transcutaneous carbon dioxide ($PtcCO_2$) monitoring in early detection of respiratory failure in ALS.

**Methods:** This is a prospective observational cohort study consisting of 50 consecutive patients with ALS. The participants underwent 3 monthly assessments for symptoms of respiratory failure, FVC and $PtcCO_2$ monitoring, until respiratory failure was clinically suspected. The presence of respiratory failure was confirmed with an overnight capnometry.

**Results:** Symptoms of respiratory failure were the most powerful tool, alerting the physician to the possibility of respiratory failure. All the patients where respiratory failure was confirmed on overnight capnometry had symptoms of respiratory failure. Shortness of breath on exertion was the most common symptom, present in 74% of the patients with confirmed respiratory failure. 37% of these patients had FVC of $>$ 50% predicted and only 15% had day time hypercapnia ($PtcCO_2 >$ 6.0 kPa). None of the patients had day time hypercapnia without any other marker of respiratory failure. There was statistically significant difference between the day time $PtcCO_2$ and median overnight $PtcCO_2$ ($p = 0.0002$).

**Discussion and conclusion:** This study has emphasized the importance of symptom history. All the patients who were suspected to be in respiratory failure on the basis of symptoms were confirmed to have significant nocturnal hypoventilation on overnight capnometry. Once again, the limitations of FVC in predicting respiratory failure are demonstrated in this study. A normal daytime $PtcCO_2$ may be falsely reassuring as most patients with symptoms of respiratory failure and nocturnal hypercapnia, had a normal daytime $PtcCO_2$ and there was a significant difference in the daytime and nocturnal $PCO_2$ levels. Day time hypercapnia is a late finding and confirms established respiratory failure. Based on the most common symptoms of respiratory failure reported by the participants we were able to modify the initial questionnaire. More work is required for validation of the final questionnaire and to determine the cut-off score, likely to have strong positive-predictive-value in diagnosing respiratory failure.

**Acknowledgements:** The Motor Neurone Disease Association (UK), Sheffield Teaching Hospitals NHS Trust and our patients.

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## C63 INDEPENDENT FACTORS ASSOCIATED WITH FAILED USE OF NONINVASIVE VENTILATION (NIV) IN PATIENTS WITH ALS/MND

CAZZOLLI P[1], BROOKS BR[2], LEWARSKI J[3], MCKIM D[4], NAKAYAMA Y[5], CHATBURN R[6]

[1]*ALS Care Project, Canton, Ohio, USA,* [2]*Carolinas Neuromuscular/MDA-ALS Center, Charlotte, North Carolina, USA,* [3]*Invacare Corporation, Elyria, Ohio, USA,* [4]*The Ottawa Rehabilitation Centre, Ottawa, Ontario, Canada,* [5]*Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan, Japan,* [6]*Cleveland Clinic Foundation, Cleveland, Ohio, USA*

*Email address for correspondence: pcazzollirn@aol.com*

*Keywords: noninvasive ventilation (NIV), oral secretions, upper airway maintenance*

**Background:** Patients with ALS/MND may tolerate noninvasive ventilation (NIV), unless oral secretions become severe. Progression of swallow and cough impairment in bulbar ALS may result in frequent/constant collection of mucus and/or saliva at the back of throat, precluding noninvasive upper airway maintenance and NIV tolerance.

**Objective:** To investigate possible factors, independent from oral secretions, that may cause failed NIV use.

**Methods:** In this observational study, 157 consecutive ALS/MND patients were followed prospectively at the start of NIV and for length of survival during subsequent home visits. A validated Oral Secretion Scale was used to measure the level of oral secretions (saliva and mucus), which ranged from normal, minimal to moderate, and severe to most severe. Tolerance of NIV, ambulatory status, medications, and use of NIV prior to death were assessed.

**Results:** At the start of NIV, 26% of patients (41/157) began NIV during emergency hospitalizations, while waiting for pulmonary appointments, despite acute respiratory failure (ARF) signs. Of the 41, 46% (19/41) survived, in which 12 were non bulbar and continued NIV. Of those that didn't continue with NIV, 15% (6/41) began tracheostomy invasive ventilation (TIV) after continuous positive airway pressure (CPAP) was attempted and failed. 16 others, in which 76% (12/16) had severe oral secretions, either died or began TIV after failed use of NIV during emergency ARF.

A total of 86% (135/157) continued NIV. A subset of 12% (16/135) these NIV nonbulbar patients used NIV for 24 hours per day for 24 to 99 months (mean of 50.5 months). When NIV was initiated, 50% (68/135) were independently ambula-

tory or walked with help, 13% (17/135) presented with a respiratory onset, and 56% (76/135) were nonbulbar and initially tolerated NIV. Of the 135, 29 nonbulbar had unexpected ARF, while off NIV, including 1 accidental withdrawal. Of these, 83% (24/29) died and 17% (5/29) began TIV. 45%(13/29) were also ambulatory.

In those nonbulbar patients in whom NIV was tolerated, 10% (13/135) survived up to 99 months. At this point, these patients withdrew from NIV, anticipating death to occur, as they desired. 7% (10/135) were given morphine sulphate at a hospice and became intolerant of NIV. 4% (5/135) of these NIV users reported sudden inability to breathe, while using NIV, despite no alteration of the NIV settings, these patients either died or began TIV. A small percentage of nonbulbar, NIV users 3% (4/135) began TIV after CPAP intolerance.

**Discussion and conclusion:** Factors independent from excessive oral secretions may be associated with failed NIV use and cause unexpected deaths or unplanned TIV include: Delay in NIV initiation until pending ARF; use of CPAP or bilevel ventilators with spontaneous mode; unawareness of pending ARF and need to use NIV, particularly in ambulatory and respiratory onset patients; use of morphine in successful NIV users because of hospice protocols; and if settings not adjusted as respiratory status changes.

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## C64 FURTHER ANALYSIS: DIAPHRAGM PACING IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS WITH CHRONIC HYPOVENTILATION

KATZ J[1], MOORE D[1], IGNANI A[2]

[1]*CPMC, San Francisco, CA, USA,* [2]*Synapse, Cleveland, OH, USA*

*Email address for correspondence: katzjs@sutterhealth.org*

*Keywords: diaphragm pacing, hypoventilation, respiratory*

**Objectives:** To study survival in ALS patients, undergoing diaphragm pacing.

**Background:** We have performed further analysis of the original 106 patient study of DPS in ALS. Here, we focused on patients who fulfilled the newer Humanitarian Device Exemption criteria and compared their outcomes to historical controls.

**Methods:** We tested survival data between three ALS cohorts: DPS, a retrospective analysis by Lechtzin et al. examining the NIV therapy in ALS, and placebo subjects from the multi-centre study of minocycline. We used uniform criteria, selecting only subsets of patients with: less than three years since onset; FVC below 85% at enrolment; and FVC above 45% at three months post enrolment. Thus, 48 DPS-3yr patients, 51 Lechtzin-3yr and 92 mino-3yr patients were analysed. We used standard statistical methods to compare survival rates, from onset of disease and time since diagnosis. We corrected for effects of significant covariates on survival (age, gender, onset location, FVC, riluzole use, ALSFRS-r, FRS preslope, and observed FRS and FVC declines during the studies).

**Results:** Median survival from the onset of disease was $38.8 \pm 3.4$ months (95% CI 36.3 to 49.3) for DPS-3yr, $32.5 \pm 1.6$ months (29.0 to 41.0) for Lechtzin-3yr, and $31.2 \pm 2.4$ months (CI 27.1 to 34.9) for the mino-3yr cohort. Median survival from diagnosis was 33.5 (27.3 to 38.8) for DPS; 22.4 months (19.2 to 26.3) for Lechtzin-3yr study; 18.2 months (14.9 to 24.7) for mino-3yr. These were significant for DPS versus the other two groups and remained significant after adjustments.

We found differences ($p < 0.05$) between the 3-yr groups only in baseline ALSFRS-r and Riluzole use. The predicted FVC was highest for the mino-3yr group (this study included patients with FVC above 75% at baseline). Age and ALSFRS-r preslope affected survival but neither factor differed significantly among study groups.

**Discussion and conclusion:** This historical analysis found roughly six months improvement in overall survival from disease onset in the DPS-3yr patients. This was greater when comparing survival time since diagnosis. These are less than the 20 month survival difference suggested by earlier work, which likely reflects limiting subjects to less than three years disease duration and controlling for baseline pulmonary function. Despite baseline similarities between the three patient groups, other differences were difficult to correct. In particular, the specific respiratory phenotype in the DPS cohort required a 'stimulatable diaphragm', in contrast to the other two groups, which could itself affect survival. We also cannot exclude other factors, such as overt dyspnea or simple appearance could have influenced decisions about who underwent surgery. Whilst several ongoing randomized trials should answer remaining questions, our analysis provides further rationale for the continued study of diaphragm pacing.

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## C65 PALLIATIVE THERAPY DURING WITHDRAWAL OF VENTILATION – A RETROSPECTIVE ANALYSIS OF A 10 YEARS EXPERIENCE IN ALS

KETTEMANN D, MAIER A, MÜNCH C, MEYER R, FUNKE A, MEYER T

*Charité – Universitätsmedizin Berlin, Neurologische Hochschulambulanz, Ambulanz für ALS und andere Motoneuronenerkrankungen, Berlin, Germany*

*Email address for correspondence: andreas.funke@charite.de*

*Keywords: palliative care, end of life, ventilation withdrawal*

**Background:** Non-invasive (NIV) and invasive ventilation (IV) are established treatment options in ALS. Over the course of the disease, a group of ALS patients decided for the elective termination of ventilation (ETV). Withdrawal of ventilation based on a patient's consistent willing is in conformity with the German law and medical ethics. However, there are few systematic studies on palliative measures during ETV.

**Methods:** Ventilation withdrawal was performed in 41 ALS patients (September 2002 to March 2014). In ETV, 2 palliative concepts were differentiated: 1) Intensified symptom control (ISC), in patients with ventilation-free periods and anticipated minor dyspnoea following ETV (with benzodiazepines = BZD; injectable morphine sulphate = MSI; 2) Deep sedation (DS), in patients without ventilation-free time and anticipated high-grade dyspnoea following ETV (BZD, MSI, propofol).

**Results:** ETV was realised in patients with NIV in n = 12 (29.3%), and with IV in n = 29 (70.7%). Gender distribution was 15:27 (F: M). Median age was 59.8 years (35–84). Mean ALS-FRSr was 15.6/48 (NIV), and, respectively, 4.6/48 (IV). Five patients (11.9%) presented with incomplete ophthalmoplegia and n = 2 (4.8%) had a complete Locked-in Syndrome. The median course of ventilation up to ETV was 15.1 months (0.03–53). Mean daily ventilation time was 22.3/24h (NIV) and 23.2/24h (IV). ISC was administered to 20/38 patients (52.6%), and DS to 18/38 patients (47.4%). DS was the predominant palliative concept for IV (n = 16; 88.8%). In ISC, the median MSI dose was 626 mg (32 - 3.145 mg). The median duration to asystole (time from removal of the ventilation mask or, respectively, disconnection from the respirator to asystole) was 33 h: 43 min (164 h: 45 min - 00 h: 27min). In DS, the median dose for MSI was 178 mg (13–850 mg) and for propofol 438 mg (66 - 1.133 mg). The median duration to asystole was 15.6 min (09–38 min).

**Discussion and conclusion:** ALS patients with either IV or continuous NIV requested ETV. Patients with ophthalmoplegia were overrepresented in this patient group. Palliative therapy by means of ISC and DS provided sufficient symptom control in conjunction with ETV. However, the quality of life and the burden of care around the decision making process and during the disease course close to death are largely unknown and urgently need to be studied.

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## C66 WITHDRAWAL OF VENTILATION AT THE REQUEST OF A PATIENT WITH MND: EXPLORING EXPERIENCES OF THOSE INVOLVED

PHELPS K[2], REGEN E[2], GARNER I[1], OLIVER D[3], MCDERMOTT CJ[4], FAULL C[1, 5]

[1]*LOROS Hospice care for Leicester, Leicestershire and Rutland, Leicester, UK,* [2]*Leicester University, Leicester, UK,* [3]*University of Kent, Kent, UK,* [4]*University of Sheffield, Sheffield, UK,* [5]*DMU-LOROS Centre for the Promotion of Excellence in Palliative care, De Montfort University, Leicester, UK*

*Email address for correspondence: christinafaull@loros.co.uk*

*Keywords: ventilation withdrawal, end of life*

**Background:** Very little is known about the withdrawal of ventilation at the request of a patient who has become dependent on it. Whilst working with doctors in palliative care in the UK, we found a high degree of ethical, practical and emotional challenges and an absence of guidance for this area of care (1). The NICE guidance on the use of NIV in MND in the UK suggests interviewing the professionals involved in such events as a focus of research (2).

**Objectives:** To compare and contrast the experiences of bereaved family, doctors and other healthcare professionals involved in withdrawing ventilation at a patient's request.

**Methods:** A qualitative, exploratory approach using one-to-one in-depth interviews with a close family member and health care professionals who have had this experience in the last five years. Interview transcripts were analysed thematically using a grounded theory approach.

**Results:** 17 relatives, 24 doctors and 26 other health professionals (HPs) participated from 20 sites across the England and Wales. Participants reflected on the stories of more than 42 patients.

The emotionality and the tensions of the situation were especially vivid for all. The logistics were more variably recalled but both families and HPs held some technical aspects in great detail.

Families described a long journey to the point of decision, often triggered by loss of communication or overwhelming sense of dependence or loss of self-determination. Families often spoke of patients choosing to end life.

Families often sensed that professionals were inexperienced, illustrated by an absence of clear information sharing and a lack of choice.

HPs may know the patient and family well or be called upon to deliver the care with little or no previous involvement.

Nurses spoke of advocacy for the patient and the family. Some felt uneasy about the decision and the withdrawal itself. They often felt professionally vulnerable.

The clarity for the doctors of the ethical and clinical decision-making was in contrast to the multi-layered and conflicting feelings they experienced in carrying out the patient's wishes. Medical indemnity organizations appeared unclear about the professional and legal acceptability of this and this increased the complexity and the stress of the situations.

**Discussion and conclusion:** This is a complex area of care and most HPs are novices. Those HPs that have had more experience or who are supported by HPs who have are better able to guide families and colleagues. Mentoring and other systems need to be developed to support those involved and improve patient outcomes.

**Acknowledgements:** This work was funded by the MND Association (UK).

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# SESSION 8C BIOMARKERS (II)

## C67 PHENOTYPIC CHARACTERIZATION AND PREDICTION OF DISEASE PROGRESSION IN ALS PATIENTS USING A METABOLOMICS APPROACH

BLASCO H[1,2], NADAL-DESBARATS L[2,6], PRADAT P-F[3], GORDON PH[3], MADJIHOUNOUM B[2], PATIN F[1], MOREAU C[4], DEVOS D[4], VEYRAT-DUREBEX C[2], MAVEL S[2,6], EMOND P[2,6], ANDRES CR[1,2], CORCIA P[2,5]

[1]*CHU Tours, Tours, France,* [2]*INSERM U930, Tours, France,* [3]*Fédération des Maladies du Système Nerveux, Centre Référent Maladie Rare SLA, Hôpital de la Pitié-Salpétrière, Paris, France,* [4]*Service de Neurologie, Lille, France,* [5]*Centre SLA, service de Neurologie, Tours, France,* [6]*Programme Pluri formation, Tours, France*

*Email address for correspondence: helene.blasco@univ-tours.fr*

*Keywords: metabolomics, prediction, disease progression*

**Background:** Markers of endophenotypes are missing in ALS and may be useful to plan clinical trials. Although numerous clinical features have been linked to the prognosis, they are not used in practice. So far, no study has used the combination of biological and clinical parameters to perform prognosis prediction.

**Objectives:** To assess the metabolome profile of cerebrospinal fluid (CSF) from ALS patients, to (i) explain the clinical characteristics at diagnosis and to (ii) predict the evolution of the disease in a separate cohort after combination of clinical and biological parameters.

**Methods:** CSF samples from ALS patients were analysed by $^1$H-NMR spectroscopy. The following clinical parameters were collected at diagnosis: site at onset; age at onset; weight loss at diagnosis; BMI; ALSFRS; and FVC. The 3 following parameters were used as markers of disease evolution: change in ALSFRS (var_ALSFRS); changes in weight (var_weight) over one year; and survival. Two cohorts (training cohort: $n = 49$; and test cohort: $n = 25$) were established. An independent OPLS-DA model was established from metabolomics signature of both cohorts to explain the clinical parameters at diagnosis and the markers of disease evolution, and any common metabolites were noted. Following this a multivariate model from the training cohort, including relevant clinical parameters and metabolomics data, was used to predict var_ALSFRS and var_weight in the test cohort, using a ROC curve. The same strategy was used to predict survival of patients in the test cohort, using a parametric survival analysis.

**Results:** The OPLS-DA models explaining the clinical parameters at diagnosis or the disease evolution revealed correct performance in the both cohorts, with between 2 and 6 common metabolites, involved in branched amino acid and glucose metabolism, or oxidative stress. The ROC curve predicting var_ALSFRS used metabolomics data, FVC, site at diagnosis and weight loss at diagnosis and enabled a correct classification of 72% of patients in test cohort. Similarly, the prediction of var_weight using metabolomics data, gender, FVC and site at onset and showed a correct classification in 70.8% of patients in the test cohort. The best model to predict survival including metabolomics data, diagnosis delay and site at onset revealed a correct prediction for 76% of patients. Importantly, models including metabolomics data or clinical parameters alone provided worst results.

**Discussion and conclusion:** The analyses showed that the CSF metabolome can be used to explain the endophenotypes at diagnosis, disease evolution and to perform disease prediction. To our knowledge, this is the first metabolomics study to use separate cohorts to predict disease progression, after inclusion of relevant from biological and clinical parameters.

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## C68 SMADS AS MUSCLE BIOMARKERS IN AMYOTROPHIC LATERAL SCLEROSIS

SI Y[1], CUI XQ[1], KIM S[1], WIANS R[1], SORGE R[1], OH S[1,2], AL-SHARABATI M[1], LU L[1,2], CLAUSSEN G[1], ANDERSON T[1], YU S[1], MORGAN D[1], KAZAMEL M[1], KING P[1,2]

[1]*University of Alabama, Birmingham, AL, USA,* [2]*Birmingham VA Medical Center, Birmingham, AL, USA*

*Email address for correspondence: pking@uab.edu*

*Keywords: muscle, Smads, biomarkers*

**Background:** There is a strong need for biomarkers in ALS that can assist with the diagnosis and/or monitoring of disease progression. Changes occur in skeletal muscle at the very earliest stages of ALS providing the rationale for investigating molecular signatures in muscle that could serve as biomarkers. Using RNA sequencing of ALS and control muscle biopsy samples, we previously reported the identification of targets unique to ALS muscle.

**Objective:** To validate Smad 8, a target that was identified by RNA sequencing of ALS muscle, as a biomarker of disease.

**Methods:** Total RNA was extracted from muscle biopsy samples of 27 ALS patients and 33 controls (13 normal, 11 myopathy, 9 neuropathy). Smad mRNA was quantitated by real time PCR (qPCR) using GAPDH as an internal control. For validation in the ALS mouse, gastrocnemius muscle samples from G93A SOD1 mice (Bl6 background) and littermate controls were harvested at different ages and assessed for Smad expression by qPCR, Western blot and immunohistochemistry. Spinal cord and brain tissues were also analysed. Smad expression was assessed in a mouse model of sciatic nerve injury to determine the specificity and reversibility of induction.

**Results:** Smad 8 mRNA was significantly elevated in human ALS muscle samples by 3–5 fold over diseased controls ($P < 0.0001$). Smad 1 and 5 were elevated to a much lesser extent but still greater than controls ($P < 0.05$). A similar pattern of induction was seen in the ALS mouse starting at preclinical stages, with increases in mRNA and protein expression paralleling disease progression. No Smad induction was detected in spinal cord or brain tissues. Phosphorylation (activation) of Smads also significantly increased at all stages ($P < 0.0001$) and paralleled disease activity. Immunohistochemistry of muscle samples indicated an accumulation of Smad protein with disease progression. In the sciatic nerve injury model, Smads were equally induced during the acute denervation stage compared to sham controls ($P < 0.05$), and normalized during the reinnervation phase.

**Discussion and conclusion:** Smads are muscle biomarkers of disease progression in ALS at the mRNA, protein and post-translational levels, with Smad 8 mRNA possibly being a unique molecular signature. The reversibility of induction upon reinnervation in a sciatic nerve injury model suggests that the Smads could be a marker of disease regression and thus enhancing their potential utility in clinical trials.

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## C69 MOTOR UNIT NUMBER INDEX (MUNIX): READY FOR CLINICAL ALS TRIALS – A 15 MONTHS LONGITUDINAL MULTICENTRE TRIAL

NEUWIRTH C[1], BARKHAUS PE[2], BURKHARDT C[1], CASTRO J[3], CZELL D[4], DE CARVALHO M[3], NANDEDKAR S[5], STÅLBERG E[6], WEBER M[1,7]

[1]*Neuromuscular Diseases Unit, Kantonsspital St. Gallen, St. Gallen, Switzerland,* [2]*Milwaukee Veterans Administration Medical Center and Medical College of Wisconsin, Milwaukee, Wisconsin, USA,* [3]*Neuromuscular Unit, Department of Neurosciences, Hospital de Santa Maria, and Instituto de, Lisbon, Portugal,* [4]*Kantonsspital Winterthur, Department of Neurology, Winterthur, Switzerland,* [5]*CareFusion, Middleton, Wisconsin, USA,* [6]*Department of Clinical Neurophysiology, University Hospital, Uppsala, Sweden,* [7]*Department of Neurology, University Hospital Basel, Basel, Switzerland*

*Email address for correspondence: christoph.neuwirth@kssg.ch*

*Keywords: MUNIX, biomarkers, ALSFRS-R*

**Background:** Motor Unit Number Index (MUNIX) is a novel neurophysiological measure that provides an index for the number of motor neurons in a muscle (1, 2) and is an ideal candidate to track lower motor neuron loss in ALS patients.

**Objective:** To investigate MUNIX in a set of muscles, in ALS patients, in a longitudinal multicentre setting to evaluate its sensitivity as a marker for disease progression in comparison to functional decline, as represented by ALSFRS-R.

**Methods:** Between 07/2010 and 01/2014 three study centres applied the MUNIX technique in 48 ALS subjects over 15 months. Six muscles (biceps brachii (BB); abductor digiti minimi (ADM); abductor pollicis brevis (APB); tibialis anterior (TA); extensor digitorum brevis (EDB); abductor hallucis AH) were measured in each subject on the clinically less affected side of the body, every 3 months. Decline of MUNIX and ALSFRS-R was compared.

**Results:** MUNIX was easy to perform and well tolerated. Out of 48 patients, 38 reached a follow-up visit at month 12. The muscle-specific intraclass correlation coefficient (ICC) showed very good reproducibility (Intra-rater reliability between 0.81 and 0.97, mean 0.89, Inter-rater reliability 0.46 and 0.92, mean 0.80). The relative decline of MUNIX differed between muscles and was different between subgroups of subjects with bulbar, lower and upper limb onset. For all subjects, ALSFRS-R declined at a rate of 2.3% per month. MUNIX of AH and BB declined at a similar rate (2.4% and 2.6%). Other muscles declined at higher rates between 3.3% and 4.2% and were statistical significant at several points in time ($p\ 0.05 > 0.002$). Using the total score of MUNIX (either of all 6 or of the 4 muscles excluding AH and BB), MUNIX 6 declined significantly with 3.2% decline per month, and MUNIX4 with 3.7% per month ($p\ 0.03 > 0.0005$). Subgroup analysis revealed different rates of decline in ALSFRS -R for bulbar onset subjects (2.8% per month, $n = 17$) and lower (2.1% per month, $n = 15$) or upper limb onset (1.9% per month, $n = 16$), while MUNIX4 and MUNIX6 showed similar decline rates across all subgroups (MUNIX 4: 3.6% to 3.8% per month, MUNIX6: 3.1% to 3.4% per month).

**Discussion and conclusion:** MUNIX measurements in multiple muscles reveal a good inter- and intra-rater reliability for detecting decline in ALS subjects. MUNIX decline significantly exceeded decline of ALSFRS-R in several muscles in spinal onset ALS subjects and is similar to ALSFRS-R decline in bulbar onset ALS subjects. While ALSFRS-R decline differs in different onset subgroups, MUNIX total scores reveal the same decline rates in all subgroups. Consequently, MUNIX is a reliable electrophysiological biomarker to track the underlying disease process of lower motor neuron loss in ALS.

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## C70 DIAGNOSTIC UTILITY OF THRESHOLD TRACKING TRANSCRANIAL MAGNETIC STIMULATION IN ALS – STARD STUDY

GEEVASINGA N, MENON P, YIANNIKAS C, KIERNAN MC, VUCIC S

*University of Sydney, Sydney, NSW, Australia*

*Email address for correspondence: nimesh84@hotmail.com*

*Keywords: diagnostic test, transcranial magnetic stimulation, STARD criteria*

**Background:** Early and reliable diagnosis of amyotrophic lateral sclerosis (ALS) is essential for effective therapeutic and symptomatic interventions, thereby improving the quality of patient care. The technique of transcranial magnetic stimulation (TMS) with the potential to detect preclinical upper motor neuron (UMN) involvement, may aid in facilitating an early and reliable diagnosis of ALS.

**Objective:** The present study prospectively assessed the diagnostic utility of threshold tracking, paired pulse TMS, in ALS as per the guidelines of the standards of reporting of diagnostic accuracy (STARD) criteria.

**Methods:** Two hundred and seventy one patients were prospectively recruited and underwent threshold tracking TMS studies. 63 of these patients were diagnosed as having other neuromuscular disorders. The diagnosis of ALS was made in the remaining 208 patients, according to the Awaji criteria. Of these patients, 32 patients had an inexcitable motor cortex limiting further TMS studies.

**Results:** The mean value of short interval intracortical inhibition (SICI) (1–7 ms) and peak value (3 ms) were significantly reduced in the ALS group ($P < 0.0001$) along with reduced cortical silent period duration ($P < 0.005$) and increased motor evoked potential amplitude ($P < 0.05$).

Receiver operating curve (ROC) analysis for mean SICI between the ALS and other neuromuscular disorder group revealed an area under the curve (AUC) of 0.74. A SICI cut-off value of 6.7% had a sensitivity of 70% and specificity of 71% (+ LR 2.3, -LR 0.7), in differentiating ALS from other neuromuscular disorders. The diagnostic utility was maintained in the AWAJI clinically probable /possible ALS group (AUC 0.73, diagnostic SICI cut-off 6.7% with sensitivity 69%, specificity 71%, + LR 2.3 -LR 0.4).

**Discussion and conclusion:** Cortical hyperexcitability as evidenced by TMS testing was confirmed to be a feature of ALS. By utilising the paired pulse threshold tracking technique TMS, the parameter of SICI was established as a reliable diagnostic test in differentiating ALS from other mimic neuromuscular disorders.

The threshold tracking TMS technique could complement the current diagnostic criteria and aid in the earlier recruitment of patients into clinical treatment trials and earlier commencement of Riluzole.

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## C71 STRUCTURAL CONNECTOME ANALYSIS IN ALS AT MULTICENTER LEVEL: A CONTROLLED STUDY IN 200 PATIENTS

MÜLLER H-P[1], ABRAHAMS S[2], BEDE P[3], FILIPPI M[4], AGOSTA F[4], GOVIND V[5], GROSSKREUTZ J[6], PRUFLO J[7], TURNER MR[8], KASSUBEK J[1], ON BEHALF OF THE NEUROIMAGING SOCIETY IN ALS (NISALS)[1]

[1]*Dept. of Neurology, University of Ulm, Ulm, Germany,* [2]*Psychology – PPLS, Euan MacDonald Centre for MND Research, The University of Edinburgh, Edinburgh, UK,* [3]*Academic Unit of Neurology, Trinity College Dublin, Dublin, Ireland,* [4]*Neuroimaging Research Unit, Institute of Experimental Neurology, Division of Neuroscience, San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan, Italy,* [5]*Department of Radiology, University of Miami School of Medicine, Miami, Florida, USA,* [6]*Hans-Berger Department of Neurology, Jena University Hospital, Jena, Germany,* [7]*Department of Neurology, University of Rostock and DZNE, Rostock, Germany,* [8]*University of Oxford Nuffield Department of Clinical Neurosciences, John Radcliffe Hospital, Oxford, UK*

*Email address for correspondence: hans-peter.mueller@uni-ulm.de*

*Keywords: diffusion tensor imaging, multicenter, fractional anisotropy*

**Introduction:** Diffusion tensor imaging (DTI)-based metrics is increasingly used for analysing ALS-associated white matter alteration patterns and was included in the NiSALS neuroimaging concept (1). The objective of this multicenter study was to assess structural connectivity in ALS in a large sample size to address the challenges of DTI data analysis from multiple study sites.

**Methods:** Four-hundred and seven DTI data sets from patients with ALS (N = 239) and controls (N = 168) were collected from 8 study centers (Dublin, Ireland; Edinburgh, UK; Jena, Germany; Miami, US; Milan, Italy; Oxford, UK; Rostock, Germany, Ulm, Germany). Data were obtained by different magnetic resonance imaging (MRI)-systems and by different DTI-protocols. The minimum number of data sets per site was 15 ALS patients and 10 controls. In a first step, comparability of data with the aim of pooling was tested. Therefore, a statistical analysis of fractional anisotropy (FA) in predefined regions of interest (ROIs) (ie regions that are prone to be affected in ALS as well as regions that are probably not affected in ALS) was performed in controls' data. Statistical comparisons in terms of average FA-values (2) were performed for the controls' groups of the different centers. The same approach was then applied to the corresponding ALS patient subgroups of the different centers. All analyses were performed by use of the Tensor Imaging and Fiber Tracking (TIFT) software (Department of Neurology, University of Ulm, Germany).

**Results:** Data collection has been completed and data quality control has been successfully performed for all data sets, resulting in 359 DTI data sets (201 ALS patients and 158 controls) useful for this study, ie, 48 data sets had to be excluded. As a first result, all data samples of all centers showed a characteristic pattern (FA decrease along the corticospinal tracts) for comparison at the group level.

**Discussion and conclusion:** This large-scale multicenter study is a NiSALS project intended to investigate the feasibility of solutions to challenges in the process of pooling MRI data recorded at various study centers. This approach is of utmost importance in order to establish MRI-based techniques as read-outs both for natural history assessment and for potential upcoming disease-modifying multicenter studies in ALS.

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# SESSION 9A MODULATING SOD1 TOXICITY

## C72 TREATMENT FOR AMYOTROPHIC LATERAL SCLEROSIS USING AAV9 ENCODING A MICRORNA AGAINST SOD1

STOICA L[1,2], MUELLER C[2], SALAMEH J[1], BROWN R[1], SENA-ESTEVES M[1,2]

[1]*Department of Neurology,* [2]*Gene Therapy Center, University of Massachusetts Medical School, Worcester, MA, USA*

*Email address for correspondence: lorelei.stoica@gmail.com*

*Keywords: AAV, RNAi, SOD1*

**Background:** Current gene transfer and RNAi technology is an answer to developing a therapy for ALS. Recent research in SOD1 transgenic ALS mouse models has shown that reduction of the mutant SOD1 protein levels leads to a delay in disease onset and progression (1). Artificial microRNAs have been shown to be effective at *in vivo* gene knock down (2). Adeno-associated virus (AAV) vectors are able to deliver genes to all cells of the body and have a proven safety record - with several clinical trials underway and one approved therapeutic (Glybera, UniQure). Thus, RNAi gene therapy is a great choice for treatment of SOD1 ALS.

**Objectives**: To test the therapeutic efficacy of a microRNA encoded in an AAV vector in an ALS transgenic mouse model.

**Methods**: An AAV9 vector encoding a microRNA against human SOD1 (hSOD1) and a fluorescent marker, driven by a ubiquitous CBA promoter was used in this study. Transgenic mice overexpressing mutant hSOD1$^{G93A}$ on a BL6SJL background were injected bilaterally into the cerebral lateral ventricles at post-natal day 1, with the aim of restricting the vector to the central nervous system (CNS). Motor unit number estimates (MUNEs) were recorded and enervation at multiple points assessed. At the end of the study, when mice were at the humane endpoint, hSOD1 mRNA levels were quantified and motor neurons, AAV transduction, neuromuscular junction enervation, astroglia reactivity were assessed using histology.

**Results**: Both motor neurons and astrocytes were transduced, and hSOD1 mRNA was decreased by 30**%** in the spinal cord. Furthermore, there was an extension in median survival from 135 to 206 days. Animals showed a bimodal distribution regarding the cause of death. A subset of treated animals did not develop paralysis or significant motor impairment, had preservation of MUNEs and of sciatic nerve axons, but instead were euthanized due to severe body weight loss. The second subset of treated animals developed paralysis.

**Discussion and conclusion**: We were successful with our treatment and achieved a therapeutic benefit, although it is interesting that a subset of animals were euthanized due to weight loss rather than paralysis.

One potential explanation is intestinal impairment, as seen in the ALS TDP-43 mouse model (3). It is our working hypothesis that the neonatal intervention has addressed the dominant neurological phenotype in SOD1$^{G93A}$ mice, and now secondary phenotypes are being revealed that may involve peripheral organs and tissues, which could lead to mortality. In the subset of animals that were euthanized due to paralysis, the vector may have transduced peripheral organs, resulting in a suppression of the secondary phenotypes. We are currently investigating potential causes of these secondary phenotypes.

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## C73 NOVEL *IN VIVO* ACTIVE SYNTHETIC CHEMICAL CHAPERONES AS A NEW BASIS FOR ALS TREATMENT

GETTER T[1], ZAKS I[1], BEN-ZUR T[2], BARHUM Y[2], OFFEN D[2], GRUZMAN A[1]

[1]*Bar-Ilan University, Ramat Gan, Israel,* [2]*Tel-Aviv University, Tel Aviv, Israel*

*Email address for correspondence: gruzmaa@biu.ac.il*

*Keywords: chemical chaperones, drug design, intracellular targeting*

**Background:** Artificial chaperones that include polyols, trimethyl N-oxide (TMAO), phenylbutyric acids and different amino acid derivatives, have been linked to ability to reverse the mislocalization and aggregation of proteins associated with different human diseases. The most limiting factor using chemical chaperones as drugs is their very high active concentration (mM). We have synthesized chemical chaperones that primarily targeted cell organelles and areas where mutated SOD1 (among other proteins) are aggregated (ER, lysosomes and mitochondria). Refolding by chemical chaperones enabled proteolytic enzymes and proteasome system to cleave the misfolded proteins properly.

**Methods:** For the *in vitro* evaluation of chemical chaperones the mouse NSC-34 hybrid cell line was stably transfected with human wtSOD1 or mutant SOD1$^{G93A}$. Mutant SOD1 degradation, ER stress and decreased formation of SOD1 containing aggregates was estimated by western blots. Oxidative stress conditions were obtained by using glucose oxidase or glucose to stress the cells. The effects of novel chemical chaperones on the above parameters were then tested.

**Results:** Some of the chaperones exhibited a biological effect in the μM concentration range. In NSC-34 cells, following prevention of formation of SDS resistant aggregates that included high molecular weight mutant SOD, tested compounds inhibited aggregation of oxidized human SOD1. Moreover, decreasing phosphorylation of CHOP, BiP and ATF4 by tested compounds indicates that they reduced the thapsigargin induced ER stress response in SOD1$^{G93A}$ transfected NCS-34 cells. As a final test, $H_2O_2$ production was generated in NSC-34 cells by adding glucose oxidase to the medium to induce permanent oxidative stress. In these conditions, our novel chemical chaperones significantly protected cells against oxidative stress induced apoptosis. In all *in vitro* experiments n = 6.

The most *in vitro* active and potent compound (GZ-23) was subsequently evaluated in the hSOD1$^{G93A}$ transgenic mouse model of ALS (n = 18). A 10 mg/kg dose GZ-23 was administrated daily by I.P, in separate male and female groups, from

Postnatal day 40 (P40). A significant difference in body weight between treated and non-treated mice was detected by P95. At the end of the experiment (P150) the difference between treated and untreated groups was so dramatic (in neurological functions and in the body weight) that we were able to conclude that the compound causes significant delay in ALS progression.

The basic pharmacokinetic properties of the compound were tested (n = 3), using a novel HPLC method, which we developed. Levels of GZ-23 were determined in the blood after single I.P. administration, and in the brain after 5 days of daily I.P. injections. Using a fluorinated version of GZ-23, it is clear that the compound predominantly accumulates in mitochondria. Basic toxicology of the lead compound was also investigated.

**Discussion and conclusion:** Based on these results we believe that we designed a novel drug candidate for the treatment of ALS. Such unique approach (targeted chemical *in vivo* active chemical chaperones) was never reported before.

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## C74 CU-ATSM: AN EFFECTIVE TREATMENT FOR HIGH-EXPRESSING G93A-SOD1 MICE EXPRESSING THE HUMAN COPPER CHAPERONE FOR SOD1 (CCS)

BECKMAN J[1], WILLIAMS J[1], BEILBY P[1], LOPEZ N[1], LABUT E[1], ESTÉVEZ A[2], TRIAZ E[3], DIAZ P[3], BARBEITO L[3]

[1]*Linus Pauling Institute, Corvallis, OR, USA,* [2]*University Central Florida, Orlando, FL, USA,* [3]*Pasteur Institute Montevideo, Montevideo, Uruguay*

*Email address for correspondence: joe.beckman@oregonstate.edu*

*Keywords: SOD1, therapy, copper*

**Background:** No pharmaceutical treatment has extended the life of the Gurney high-expressing $SOD^{G93A}$ mouse by more than 10–15%, despite a million dollar prize being offered for extension of life by 25%. The copper (II) complex CuATSM, used in humans as a PET-imaging agent, has previously been shown to extend life in two other SOD1-based ALS models by 26% and protect in four models of Parkinson's disease. Paradoxically, co-expression of the human copper chaperone for SOD1 (hCCS) accelerates death in low expressing G93A mice eight fold, with major decreases in the copper-dependent cytochrome c oxidase.

**Objectives:** To determine how well the copper-delivery agent CuATSM can protect high expressing SOD1-G93AxhCCS mice, as well as the standard G93A mouse model without hCCS, from the progression of ALS.

**Methods:** The mouse trials used the same male G93A breeders from Jackson to produced randomized matched treatment groups. To produce hCCS expressing mice, homozygous female mice on the same hybrid background were mated to G93A males. Mice were treated with CuATSM applied dermally twice a day.

**Results:** CuATSM (100 mg/kg/day) started at 50 days extended survival of G93A mice by 22% ($p < 0.001$, n = 23, 4 treated and 2 controls died from GI complications at ~100 days). Treatment started at birth extended life by 29% (n = 13, no mice lost). With coexpression of hCCS, all G93A-expressing mice died between 8–12 days. CuATSM treatment (12 mg/kg/day) started at 5 days rescued these pups. Of 5 kept on treatment, 4 died between 230–310 days of motor neuron disease and one mouse is still alive after one year. Measurement of SOD in spinal cord by mass spectrometry shows that 3x more $SOD^{G93A}$ (425 micromolar with copper and zinc fully bound) exists in the ventral gray matter than in the standard G93A mice (120 micromolar with half missing copper). A second cohort (n = 17) treated from birth to match the 29% survival group (G93A alone) is now 220 days old with all mice showing only slight symptoms. If CuATSM is withdrawn from the CCSxG93A mice, the animals develop motor neuron disease in 2 months. Progression could be stopped by resuming treatment with CuATSM.

**Discussion and conclusion:** CuATSM is the most effective treatment so far in the $SOD^{G93A}$ mice. CuATSM likely protects G93A mice co-expressing CCS by completing the maturation of SOD to its mature form containing copper and zinc. All humans ALS patients likely express CCS, making this model closer to the human condition than the standard ALS model. CuATSM is remarkably nontoxic and is in use in humans now.

**Acknowledgements:** We thank Drs. Son and Elliott for providing the CCS mice.

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## C75 SMALL MOLECULES THAT BLOCK PROPAGATION OF SOD1 MISFOLDING IN LIVING CELLS

AIREY D[1], GRAD L[1], VIEIRA F[2], CASHMAN N[1]

[1]*University of British Columbia, Vancouver, BC, Canada,* [2]*ALS Therapy Development Institute, Cambridge, MA, USA*

*Email address for correspondence: lgrad@mail.ubc.ca*

*Keywords: small compounds, protein misfolding, SOD1*

**Background:** Mutant SOD1 can confer its misfold on wild-type (WT) SOD1 inside living cells (1); the propagation of misfolding can also be transmitted intercellularly over multiple passages (2). Strikingly, the induction of misfolding by a mutant SOD1 template is restricted to a single tryptophan (Trp) at position 32 of the protein (1) indicating a possible point of contact between the converting and converted protein species. Two compounds, 5-fluorouridine, a chemotherapy agent, and isoproterenol, used to treat bradycardia, were originally identified as stabilizers of native dimeric SOD1 (3). Crystal-structure analysis demonstrates that both compounds bind at or near Trp32 (4). Given the importance of Trp32 for template-directed SOD1 misfolding, we hypothesized that small molecules binding at or near the Trp32 site will block SOD1 template-directed misfolding within the cell environment, thereby mitigating the spread of pathological SOD1 and potentially halting disease progression.

**Methods:** We have developed a human cell transfection-conversion system in which conditioned media from transfected cells expressing mutant or misfolded WTSOD1 is able to induce misfolding in WTSOD1 in fresh untransfected cell cultures. To test the efficacy of small molecules on their ability to block propagated SOD1 misfolding, conditioned media were treated with various small molecules prior to incubation on untransfected cells. Treated cells were then examined for misfolded WTSOD1 content either via immunofluorescence microscopy or quantitative immunoprecipitation (IP) utilizing antibodies specific for misfolded SOD1.

**Results:** The addition of 1.5 mM 5-fluorouridine to our propagated SOD1 misfolding cell culture assay revealed a decrease in the detection of induced SOD1 misfolding by 83.7% ($n = 3$; $p = 0.004$) using quantitative IP compared to untreated. Similar results were observed for 750 μM 5-fluorouracil (a structural analogue of 5-fluorouridine), which decreased propagated SOD1 misfolding by 76.4% ($n = 6$; $p = 0.002$). Surprisingly, the natural non-toxic metabolite uridine, at a concentration of 100 μM, also showed comparable preliminary results, decreasing levels of misfolded SOD1 propagation by 81.3% ($n = 2$; $p = 0.07$).

**Discussion and conclusion:** Small molecules that are predicted to bind SOD1 at or near Trp32 significantly mitigate against the transmission of propagated SOD1 misfolding. Furthermore, structural analogues show similar effects suggesting a common structural motif may allow for efficient binding at the site of SOD1 self-recognition. Our preliminary results indicate that these molecules show promise as potential therapeutics and merit further investigation.

**Acknowledgments:** This work was supported by the Canadian Institutes for Health Research and the Allen T. Lambert Neural Research Fund. Misfolded SOD1-specific antibodies were provided by Amorfix Life Sciences Ltd.

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# SESSION 9B NUTRITIONAL ASSESSMENT AND MANAGEMENT

## C76 NUTRITION AND FUNCTIONAL ASSESSMENT IN ALS PATIENTS

GENNINGS C[1], NIEVES J[2], FACTOR-LITVAK P[2], HUPF J[3], SINGLETON J[3], SHARF V[3], GOETZ R[4], MITSUMOTO H[3], COSMOS ALS STUDY GROUP[3]

[1]*Department of Biostatistics, Virginia Commonwealth University, Richmond, Virginia, USA,* [2]*Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, New York, USA,* [3]*Eleanor and Lou Gehrig MDA/ALS Research Center, Columbia University Medical Center, New York, New York, USA,* [4]*New York State Psychiatric Institute and Department of Psychiatry, Columbia University, New York, New York, USA*

*Email address for correspondence: hm264@cumc.columbia.edu*

*Keywords: nutrition, oxidative stress, health foods*

**Background:** Previous research suggests that oxidative stress is associated with the pathogenesis of amyotrophic lateral sclerosis (ALS) and individual nutrients and dietary factors may influence this process. Patients with adequate caloric intake may have reduced morbidity and longer disease duration. However, previous studies have not evaluated whether nutrients or foods are associated with ALS severity or respiratory function at the time of diagnosis.

**Objectives:** To simultaneously evaluate various nutrients and their association with ALS severity (measured by ALSFRS-R) and respiratory function (measured by forced vital capacity, FVC) using baseline data from the multi-site COSMOS study.

**Methods:** ALS severity and respiratory function, socio-demographic variables, and food frequency questionnaire data were collected at the baseline visit in the COSMOS study. Various nutrients and food groups were grouped for analysis based on whether they were considered to be anti-oxidant/healthy or oxidants/unhealthy foods and nutrients. Weighted quantile sum (WQS) regression was used to create an empirically weighted index of nutrients and foods to determine their association with ALSFRS-R and FVC. Analyses were adjusted for covariates/confounders including patient age, gender current BMI, symptom duration and dietary calories (when not included in the WQS index).

**Results:** Baseline data were available on 302 ALS patients: 59% males with median age 63.2 years, BMI 26, symptom duration 0.94 years, with median ALSFRS 37 and FVC% = 82. Empirically weighted indices of 'good' micronutrients (eg, antioxidants, fiber, isoflavones, omega 3, cysteine, vitamin D) were positively associated with ALSFRS ($p < 0.001$) and FVC ($p < 0.001$) with 91% of the weight associated with 9 of the 18 nutrients; and 80% of the weight on 6 nutrients, respectively. The WQS index of good food groups was positively associated with ALSFRS ($p = 0.001$) and FVC ($p < 0.001$) with most of the weight associated with solid fruit, fish, poultry, nuts and seeds, beneficial oils, and certain vegetables for both outcomes with the addition of eggs for ALSFRS and yogurt for FVC. In exploratory analyses, there was a significant positive association between ALSFRS ($p = 0.016$) and 5 of 16 vitamins selected (niacin, vitamin B6, vitamin K, selenium, and glutathione). A somewhat similar index was positive and significantly associated with FVC ($p = 0.021$) with 85% of the weight on 5 components: riboflavin, vitamin E, vitamin K, and glutathione.

**Discussion and conclusion:** Our unique analysis allows for the evaluation of combinations of nutrients and food groups as compared to the typical evaluation of single nutrients. We found that foods and nutrients, typically part of a healthy diet, were associated with reduced severity of ALS at baseline.

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## C77 A PROSPECTIVE MULTI-CENTRE EVALUATION OF GASTROSTOMY IN PATIENTS WITH MND

STAVROULAKIS T, SHAW PJ, MCDERMOTT CJ

*Department of Neuroscience, Sheffield Institute for Translational Neuroscience, University of Sheffield, Sheffield, UK*

*Email address for correspondence: t.stavroulakis@sheffield.ac.uk*

*Keywords: gastrostomy*

**Background:** Gastrostomy feeding is commonly used to support MND patients with dysphagia. Although recommended by both the American Academy of Neurologists and European Federation of Neurological Societies, there is currently no robust evidence to suggest the optimal method and timing for gastrostomy insertion.

**Objectives:** Evaluation of gastrostomy practice in UK MND clinics to identify the most appropriate method in terms of safety and clinical outcomes.

**Methods:** Prospective cohort study of patients who underwent a gastrostomy in 24 MND care centres/clinics. Assessments included demographic and functional characteristics, measure of respiratory function, indices of disease progression and gastrostomy-related data.

**Results:** A clinic-based variability of gastrostomy practices due to clinician preference, method availability and patient respiratory function was demonstrated. 345 patients were recruited (45.3% female/54.7% male; 48.5% limb/51.5% bulbar; mean age 64.4 years, FVC 61.5%, weight loss 8.7% of premorbid). Gastrostomy was fitted in 323 patients. In total, 344 gastrostomies were performed (171 PEGs, 125 RIGs, 45 PIGs and 3 surgical). PIG patients were significantly frailer in terms of respiratory function, percentage of weight loss and overall clinical condition. The 30-day mortality rate following PEG, RIG and PIG was 3.1%, 3.4% and 7% respectively. The 30-day mortality risk was significantly higher for patients who had lost more than 10% of their premorbid weight. Median post-gastrostomy survival time was 11.4 months for PEG, 12 months for RIG, and 6.7 months for PIG ($p = 0.003$). Peri-procedural complication rate for PEG, RIG and PIG was 24.3%, 16.5%, and 19.0% respectively. Peri-procedural patient distress was significantly higher for PEG patients. Post-gastrostomy, pneumonia, pain and constipation were sig-

nificantly higher for PIG. Increased anxiety, fatigue, tube displacement, tube leakage, tube replacement and repeated gastrostomy were significantly higher for RIG.

**Discussion and conclusion:** PEG is preferable for patients with good respiratory function and overall clinical condition, whereas RIG and PIG are used for more frail patients with compromised breathing. Differences in 30-day mortality rates were not significant, suggesting that no one specific method is superior to another in terms of peri-procedural safety. The higher 30-day mortality rate following PIG may be attributed to the fact that this was a frailer group. This may also explain the overall post-gastrostomy survival differences. An optimal practice would be early PEG placement before patient clinical deterioration and marked weight loss, as this method allows insertion of a robust large bore tube with easier post-operational tube management. RIG is a reliable alternative when PEG is deemed too risky for patient safety, but associated with higher complications and more complex tube management, due to the smaller tubes used. PIG is a relatively safe method for placing a robust large bore tube to very frail patients who undergo gastrostomy at a late stage in the course of MND.

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## C78 MORE ON BODY MASS INDEX AND SURVIVAL IN ALS

WAY K, RUDNICKI S

*UAMS, Little Rock, AR, USA*

*Email address for correspondence: sarudnicki@uams.edu*

*Keywords: body mass index (BMI), weight, prognosis*

**Background:** Obese compared to non-obese ALS patients enrolled in clinical trials lived longer (1) and in a Japanese cohort with negligible obesity, those with smaller rates of change in body mass index (BMI) (rcBMI = BMI at diagnosis - BMI at first visit/months from disease onset) had improved survival (2). Our clinic is located in a state with one of the highest rates of obesity in the US, with obesity rate of 30.9%.

**Objectives**: To determine how premorbid obesity, obesity at diagnosis, and rcBMI impact survival.

**Methods**: A retrospective chart review of ALS patients seen from January 2001-February 2013 was carried out, with survival recorded through to April 2013. At patient's first clinic visit, BMI (BMI-1), site of onset, gender, and time to diagnosis from symptom onset were obtained. Patients reported their weight in the year prior to development of symptoms of ALS; current height was used to determine their premorbid BMI (pmBMI). Information was available on 289 patients. Obesity was defined as BMI > 30.

**Results**: 104 patients (36%) were obese premorbidly and 71 (25%) at their first clinic visit. Mean change in BMI was −2.0+/-2.8 kg/m$^2$ while the mean rcBMI was -0.21+/−0.42 kg/m$^2$/mo. There was no significant difference in rcBMI between bulbar and extremity onset patients (−0.28+/−0.42 kg/m$^2$/mo vs. −0.19+/−0.42 kg/m$^2$/mo, p = 0.1027). Patients who were under or normal weight had significantly lower rcBMI compared to those overweight or obese (−0.12+/−0.19 kg/m$^2$/mo vs. -0.25+/−0.46 kg/m$^2$/mo, p = 0.0008). There was no difference in males and females regarding mean change in BMI. Premorbid obesity did not significantly impact survival. Patients with obese BMI-1 values had significantly longer survival than non-obese patients (39 months vs. 28 months, p = 0.0202). Patients whose rcBMI was slower than -0.21 kg/m$^2$/mo lived significantly longer than those with faster rates (35 months vs. 24 months, p = 0.0001).

**Discussion and conclusion**: More rapid early loss of BMI in ALS may be a marker for worse prognosis. While bulbar symptoms may contribute to weight loss, bulbar onset disease was not associated with greater rcBMI. Obesity present at diagnosis but not premorbid obesity conferred a survival benefit, supporting the theory hyper metabolism influences survival in ALS.

**Acknowledgements:** Supported by J Thomas May ALS Fund.

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## C79 UTILITY OF SELF-REPORT PATIENT SCALES IN THE EVALUATION OF DYSPHAGIA IN INDIVIDUALS WITH ALS

PLOWMAN EK, WATTS S, TABOR L, TURNER K, ROSADO M, DOMER A, GOOCH C, RICHTER J, VU T

*University of South Florida, Tampa, Florida, USA*

*Email address for correspondence: plowman@usf.edu*

*Keywords: dysphagia, screening, aspiration*

**Background:** Dysphagia is highly prevalent in individuals with ALS. Malnutrition and aspiration pneumonia increase the risk of death by 7.7 times and contribute to 25.9% of ALS mortality (1). Therefore, early identification of dysphagic symptoms is critical. Although a number of validated dysphagia patient-rated scales are used clinically, it is not known how these relate to clinician-rated objective measures of swallowing dysfunction.

**Objectives:** 1) Evaluate the relationship between patient-rated swallowing impairment and clinician-rated measures of swallow kinematics and penetration/aspiration; 2) Evaluate the relationship between patient-rated and caregiver-rated swallowing impairment levels; 3) Determine if patient and caregiver ratings of swallowing impairment using the EAT-10 Scale differ for ALS individuals who penetrate/aspirate vs. those ALS individuals who do not penetrate/aspirate.

**Methods:** 40 ALS patients (probable or definite, by El-Escorial criteria) completed the validated Eating Assessment Tool-10 (EAT-10) and underwent a standardized videofluoroscopic evaluation. An experienced-blinded clinician completed objective kinematic and temporal physiologic swallow measures, the validated Penetration-Aspiration Scale (PAS) and the Functional Oral Intake Scale (FOIS). Spearman's Rho correlation analyses were conducted between patient-rated, caregiver-rated and clinician-rated validated outcome measures. A between groups ANOVA was performed between ALS patients who penetrated/aspirated (PAS > 3) vs. ALS patients who did not penetrate/aspirate (PAS < 2), with alpha set at 0.05.

**Results:** Patient-rated dysphagia severity (EAT-10) was significantly correlated with: PAS scores (r = 0.51, p = 0.001); pharyngeal constriction ratio (r = 0.58, p < 0.0001); oropharyngeal

transit time ($r = 0.47, p = 0.002$); and FOIS ($r = -0.72, p = 0.002$). In addition, ALS patients who penetrated/aspirated demonstrated significantly higher (worse) EAT-10 scores than those who did not, $F(1,39) = 8.82$, $p = 0.005$. Mean EAT-10 scores were three times higher in ALS patients with compromised airway protection (penetrator/aspirators) than in ALS patients with safe airway protection during swallowing. ALS caregivers and ALS patients EAT-10 scores were significantly correlated ($r = 0.83$, $p < 0.0001$) and similar to ALS patients, caregiver EAT-10 scores were significantly higher in individuals who penetrated/aspirated then in those who did not $F(1,33) = 7.12$, $p = 0.012$.

**Discussion and conclusion:** In this group of individuals with ALS, patient ratings of swallow severity (EAT-10 scores) were associated with weaker pharyngeal strength, longer oropharyngeal transit times and poorer airway safety. Both patient- and caregiver- rated EAT-10 scores were three times higher (worse) in ALS patients who penetrated or aspirated, and patient and caregiver ratings were highly correlated. The EAT-10 tool could be a useful and meaningful addition to dysphagia screening in busy multidisciplinary clinics by speech therapists and also by nursing staff for referral to speech therapy services. The ALS caregiver may also have a role in dysphagia symptom reporting which may be an important factor in rural health and telemedicine, particularly when ALS communication abilities have deteriorated.

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# SESSION 10A NON NEURONAL CELLS

## C80 THE CONTRIBUTION OF LOCAL AND SYSTEMIC INFLAMMATION TO NEURODEGENERATION

PERRY VH

*University of Southampton, Southampton, UK*

*Email address for correspondence: vhp@soton.ac.uk*

*Keywords: microglia, proliferation, inflammation*

A consistent feature of the neuropathology of diverse chronic neurodegenerative diseases is the response by the innate immune cells of the brain, the microglia and macrophage populations. The microglia take on an activated morphology and increase in number as disease progresses. Recent studies show that the molecules, CSF1 and IL-34, which drive the proliferation of the microglia, also prime the microglia so that they become more responsive to a secondary inflammatory stimulus when compared to naïve cells. The primed microglia can be switched to a tissue damaging phenotype by both a local and systemic inflammatory challenge. The data show that proliferating and primed microglia contribute to disease progression in animal models of prion disease and ALS/MND: inhibition of the CSF1R results in the delay in onset of behavioural symptoms of the disease and prolongation of lifespan. Current research is focussed on understanding the processes by which primed and proliferating microglia contribute to disease progression.

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## C81 CONTRIBUTION OF CCL2/CCR2 AXIS IN MOTOR NEURONAL PATHOLOGY OF AMYOTROPHIC LATERAL SCLEROSIS (ALS)

BENDOTTI C[1], BOSANI B[1], NARDO G[1], ROSSI M[1], SAVINO B[2,3], CARONNI N[2,3], BONECCHI R[2,3]

[1]*Dept. Neuroscience- IRCCS- Istituto di Ricerche Farmacologiche Mario Negri-, Milano, Italy,* [2]*Humanitas Clinical and Research Center, Rozzano, Italy,* [3]*Department of Medical Biotechnologies and Translational Medicine, Università degli Studi di Milano, Rozzano, Italy*

*Email address for correspondence: caterina.bendotti@marionegri.it*

*Keywords: CCL2/CCR2, transgenic mouse model, immune modulation*

**Background:** CC-chemokine ligand 2 (CCL2), one of the earliest chemokines detectable in the spinal cord of Amyotrophic Lateral Sclerosis (ALS) mouse models is the major ligand of receptor CCR2 through which it enables chemotaxis of immune cells with potential protective effect on motor neurons. On the other hand neuronal upregulation of CCL2 has been associated to increased neuronal axogenesis and motility of motor neuronal cell line in a cell-autonomous manner suggesting that CCL2 exerts functions other than chemotactic activity and is likely to be involved in neuronal plasticity. CCR2 receptor, which is critical for the recruitment of monocytes in the CNS is also highly expressed by healthy spinal neurons where it may play a role in the modulation of CCL2 induced axogenesis.

**Objectives:** This study aimed to examine in depth the contribution of CCL2/CCR2 axis in the CNS and periphery to evolution of pathology in a mouse model of familial ALS.

**Methods:** We examined the expression of CCL2 and CCR2 mRNA by RT-PCR and the cellular distribution of the relative proteins by immunohistochemistry, in the lumbar spinal cord and sciatic nerves of transgenic $SOD1^{G93A}$ mice at different disease stages. CCR2 expression in blood monocytes of $SOD1^{G93A}$ mice was also examined.

**Results:** In the lumbar spinal cord a progressive upregulation of CCL2 mRNA and protein was observed in $SOD1^{G93A}$ mice during the disease progression. CCL2 immunoreactivity was highly expressed in motor neurons at disease onset while a prevalent expression in microglia, but not astrocytes, was evident at symptomatic and advanced disease stage. CCR2 mRNA was unchanged until the symptomatic stage when it increased by 2.5 fold with respect to control mice. Immunostaining showed a selective expression of CCR2 in motor neurons of non-transgenic mice which remarkably decreased in $SOD1^{G93A}$ mice at the onset and symptomatic stage. In the sciatic nerves of $SOD1^{G93A}$ mice, CCL2 mRNA levels were increased with respect to age-matched non-transgenic mice only from the symptomatic stage and the protein was localized in either axons and Schwann cells. No changes in CCR2 mRNA levels were observed at any time during the disease course. Reduction of CCR2 positive blood monocytes was observed at the early stages during the disease progression.

**Discussion and conclusion:** An early upregulation of CCL2 in degenerating motor neurons is probably the signal for the recruitment of potentially protective CCR2 + immune cells and/or for the induction of axonal plasticity. However, this phenomenon appears to be counteracted by the downregulation of CCR2 in the peripheral monocytes and in the motor neurons. Work is in progress to understand the mechanisms underlying the down regulation of CCR2 in $SOD1^{G93A}$ mice and to investigate the effect of its overexpression on the disease course.

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## C82 CHARACTERIZATION OF INNATE AND ADAPTIVE IMMUNE RESPONSES IN THE $HSOD1^{G93A}$-MCP1-CCR2 TRIPLE TRANSGENIC ALS MOUSE

JARA J[1], FARRIS C[2], TRIMARCHI J[2], MILLER R[3], OZDINLER H[1]

[1]*Department of Neurology, Northwestern University, Chicago, IL, USA,* [2]*Department of Genetics, Iowa State University, Ames, IA, USA,* [3]*Department of Molecular Pharmacology, Northwestern University, Chicago, IL, USA*

*Email address for correspondence: ozdinler@northwestern.edu*

*Keywords: innate immunity, corticospinal motor neurons, MCP1/CCR2*

**Background:** Multiple studies have revealed the involvement of innate and adaptive immune responses, including microglia activation, astrogliosis, infiltration of T cells, and increase of cytokine/chemokine expression and secretion both in the

motor cortex and spinal cord of ALS patients, as well as in different animals models of ALS (1). Secretion of the cytokine MCP1 (monocyte chemoattractant protein-1) has been revealed in both cerebrospinal fluid and spinal cord of ALS patients and mouse models of ALS (2). It is hypothesized that increase of MCP1 within the CNS mediates recruitment of CCR2 (CC chemokine receptor 2) + monocytes which is supported by studies revealing decreased levels of CCR2 + monocytes in the blood of ALS patients (3).

**Objectives:** The purpose of this study is to understand the cellular components and the molecular basis of innate and adaptive immune response in ALS using a novel hSOD1$^{G93A\text{-}MCP1\text{-}CCR2}$ triple transgenic ALS mouse model. Our intent is not to characterise the MCP1 and CCR2 system in ALS, but rather to use their expression pattern as a bait to genetically label cells of interest. For this purpose, we purify and analyse MCP1 + and CCR2 + expressing cells, cells that are involved in innate immunity, at different stages of disease in different regions of the cerebral cortex and spinal cord where neurodegeneration is mostly observed.

**Methods:** In the hSOD1$^{G93A\text{-}MCP1\text{-}CCR2}$ mouse model, MCP1 + and CCR2 + cells are genetically labelled with mRFP (monomeric red fluorescent protein) and eGFP (enhanced green fluorescent protein), respectively. This allows for visualization and isolation based on their fluorescent character to be utilized in immunocytochemistry analysis and microarray analysis upon fluorescent activated cell sorting (FACS)-mediated purification, respectively.

**Results:** Our results reveal that MCP1 + cells belong to microglia lineage in the motor cortex at pre-symptomatic stage, and interestingly, CCR2 + cells express markers of infiltrating monocytes. Furthermore, microarray analysis at the pre-symptomatic stage reveals sets of unique genes that are upregulated and selective pathways that are activated in response to increased innate immunity.

**Discussion and conclusion:** Evaluation of the cellular identity together with transcription profile has the potential to reveal details of the molecular controls over initiation and progression of immunity in ALS, especially in different locations in the CNS. Understanding the cellular and molecular basis of initiated immunity will help identify novel therapeutic targets for building effective treatment strategies.

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## C83 INCREASED *IN VIVO* GLIAL ACTIVATION IN PEOPLE WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS)

ZÜRCHER N, LOGGIA M, LAWSON R, YASEK J, CHONDE D, IZQUIERDO D, JOHNSON-AKEJU O, CATANA C, ROSEN B, CUDKOWICZ M, HOOKER J, ATASSI N

*Massachusetts General Hospital, Boston, MA, USA*

*Email address for correspondence: natassi@partners.org*

*Keywords: PET, biomarkers, PBR28*

**Background:** Activated microglia are increased in postmortem tissue from patients with ALS (1, 2). Numerous Positron Emission Tomography (PET) ligands were developed to image activated immune cells by binding to the 18kDa translocator protein (TSPO) that is highly expressed in activated microglia. [$^{11}$C]-PBR28 PET, is a new radiotracer that binds to TSPO with 80 times higher specificity than older generation tracers (3) and can be used in PET imaging studies with ALS patients.

**Objective:** To evaluate the degree and spatial distribution of *in vivo* neuroinflammation in patients with ALS using [$^{11}$C]-PBR28 PET.

**Methods:** Eight subjects with ALS and eight age-, gender-, and binding affinity-matched healthy volunteers underwent [$^{11}$C]-PBR28 PET imaging on a Siemens 3T integrated PET/magnetic resonance (MR) scanner at Massachusetts General Hospital. Deep tendon and pathological reflexes were tested to calculate the upper motor neuron (UMN) Burden score. Standard uptake values (activity concentration per subject mass normalized to injected dose) were created for 60–90 min post radioligand injection and normalized to whole brain mean (SUVR). A whole brain between-group analysis was conducted with individual SUVR images registered to MNI space. This voxelwise analysis was conducted in FSL using an unpaired *t*-test, mixed effects and with TSPO genotype added as regressor of no interest. A priority region of interest (ROI) for the precentral gyri was selected using Freesurfer's automated parcellation. Between-group differences in ROI SUVR were assessed using Mann-Whitney. Spearman r was used to test the correlation between UMN Burden and SUVR of the precentral gyri ROI.

**Results:** Whole brain analysis revealed significantly increased [$^{11}$C]-PBR28 binding in the motor cortices and corticospinal tracts in ALS patients compared to healthy controls ($z > 2.3$, cluster corrected $p < 0.05$). There was no brain region for which the ALS group showed less [$^{11}$C]-PBR28 binding than the control group. The left motor cortex ROI analysis showed increased [$^{11}$C]-PBR28 binding ($p = 0.02$) in ALS patients ($SUVR = 1.17 \pm 0.10$) compared to controls ($SUVR = 1.10 \pm 0.05$). SUVR of the right motor cortex was positively correlated ($r = 0.74$) with the UMN Burden ($p < 0.05$). Visual evaluation of the SUVR images showed increased binding in the motor cortices in patients with limb-onset ($N = 6$) compared to patients with bulbar-onset ALS ($N = 2$).

**Discussion and conclusion:** Our findings of increased *in vivo* [$^{11}$C]-PBR28 binding in the motor cortices compliments the pathological findings of increased active microglia near motor neurons reported in post mortem studies. Further studies are needed to determine the role of [$^{11}$C]-PBR28 as a diagnostic or pharmacodynamic biomarker in ALS.

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## C84 OLIGODENDROCYTES DYSFUNCTION IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

LEUNG JYK, KING A, VICKERS J

*Wicking Dementia Research and Education Centre, University of Tasmania, Hobart, TAS, Australia*

*Email address for correspondence: ykjleung@utas.edu.au*

*Keywords: glial biology, disease progression, protein processing*

**Background:** In adulthood, oligodendrocytes undergo continuous turnover to replace damaged oligodendrocytes and myelinate new axons as part of the neural plasticity process. It has been shown that in diseases like multiple sclerosis, this turnover mechanism is disturbed and damaged oligodendrocytes are unable to be replaced by newly formed oligodendrocytes. Recently, oligodendrocyte dysfunction has been implicated in ALS and aggregates of ALS mutant proteins (both SOD1 and TDP43) have been observed in the cytoplasm of oligodendrocytes found in the spinal cord of ALS patients. An increased number of oligodendrocytes precursor cells with no change in adult oligodendrocyte number, have been observed in the spinal cord of mSOD1$^{G93A}$ mice, suggesting that the differentiation process of oligodendrocytes might be impaired in ALS. Therefore, we hypothesize that the presence of the ALS mutant proteins affects the differentiation of oligodendrocytes, which contributes to the failure in replacing damaged oligodendrocytes during disease progression, and ultimately leads to axon degeneration seen in ALS.

**Methods:** Spinal cord sections from mSOD1$^{G93A}$ and wild type mice were immunolabeled using a range of antibodies to selectively labeled oligodendrocytes at different differentiation stages, including: NG2, O4, Olig1 and CNPase.

**Results:** In comparison to the wild type, the mSOD1 mice have an increased in expression of progenitor cells marker (NG2) and marker for oligodendrocytes at pre-myelinated state (O4), and a decrease in mature oligodendrocytes markers (Olig1 and CNPase). This indicated that most of the oligodendrocytes present in mSOD1 mice were at the pre-myelinated stages. Previous study has identified a G protein-coupled receptor, GPR17, which function as a blockage for the maturation and formation of myelin in oligodendrocytes. Immunohistochemistry staining using antibody against GPR17 has revealed that majority of the GPR17 labelling co-localized with that of O4 in the grey matter of the spinal cord in mSOD1 mice, however such co-localisation was not observed in wild type.

**Discussion and conclusion:** The result indicated that the oligodendrocytes might be arrested at the pre-myelinating stages in the mSOD1$^{G93A}$ mouse model and that this may prevent it from replacing the damaged oligodendrocytes. Further *in vitro* studies using primary cell culture models should be performed to investigate the exact role that the ALS mutant proteins have in the differentiation process of oligodendrocytes.

This finding demonstrates that the presence of the ALS mutant protein affect the differentiation process of oligodendrocytes and might partly be responsible for the axon degeneration observed in ALS. This study has shown evidence supporting the potential involvement of oligodendrocytes in the disease pathogenesis of ALS.

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# SESSION 10B COGNITIVE CHANGE

## C85 IS ALS-FTD THE SAME AS FTD?

SNOWDEN J[1,2], THOMPSON J[1,2], ADAMS J[1,2], JONES M[1,2], NEARY D[1,2], MANN D[2], PICKERING-BROWN S[2]

[1]*Salford Royal NHS Foundation Trust, Greater Manchester, UK,* [2]*University of Manchester, Manchester, UK*

*Email address for correspondence: julie.snowden@manchester.ac.uk*

*Keywords: frontotemporal dementia (FTD), clinical features, cognition*

An association between ALS and frontotemporal dementia (FTD) is now well established, on clinical, pathological and genetic grounds. ALS and FTD may co-occur in a patient and within a family. Both are associated with TAR DNA-binding protein 43 (TDP-43) and more rarely fused-in sarcoma (FUS) pathology. In both, hexanucleotide repeat expansions in the C9ORF72 gene have been identified. Nevertheless, the precise relationship between ALS and FTD is not fully understood.

On the one hand, findings of cognitive and behavioural changes in ALS, which are a) graded in severity and b) qualitatively similar to those of FTD, support the notion of a continuum between ALS and FTD. On the other hand, longitudinal evidence in individual ALS patients, for a transition from mild cognitive impairment to frank FTD is limited. Moreover, the proportion of people with FTD who develop ALS is relatively small, despite a protracted illness course. People with ALS may not be equally vulnerable to developing FTD and vice versa.

In this talk I examine the relationship between ALS and FTD from the perspective of dementia. I address the heterogeneity of FTD with respect to clinical phenotype (behavioural/executive, non-fluent aphasic, semantic), type of frontotemporal lobar degeneration pathology (TDP-43, FUS and tau) and genetic mutations (C9ORF72, GRN, MAPT). I examine the position of ALS-FTD in the context of this diversity, based on our own data from several hundred patients with clinical forms of FTD and on the published literature.

Our own data show ALS to be present in around 14% of FTD cases. Patients with ALS-FTD are slightly older than FTD-only patients and have a male bias. Although each of the clinical phenotypes associated with FTD are found in ALS-FTD, pure syndromes of progressive non-fluent aphasia and semantic dementia are disproportionately rare. Retrospective analysis suggests behavioural and cognitive differences between ALS-FTD and FTD, which require further delineation by systematic, prospective investigation. Pathological examination reveals TDP-43 pathology in all ALS-FTD cases, but only around half of FTD-only cases. TDP-43 subtyping helps to distinguish ALS-FTD from FTD-only. Genetic screening for known mutations associated with FTD shows a strong association between C9ORF72 and ALS-FTD, but no association with GRN or MAPT mutations. The clinical characteristics of patients with each of these mutations are distinct.

The findings point to distinct clinical, pathological and genetic characteristics in ALS-FTD. The notion of a continuum may be apt in defining the spectrum of clinical presentations within ALS-FTD families, but not within the populations of ALS and FTD as a whole. ALS-FTD may represent a specific, aetiologically distinct variant of FTD.

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## C86 THE MEDICAL DECISION-MAKING CAPACITY IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

SPATARO R

*University of Palermo, ALS Clinical Research Centre, Palermo, Italy*

*Email address for correspondence: rossellaspataro@libero.it*

*Keywords: decision-making capacity, behavioural impairment, neuropsychology*

**Background:** The relentless progression of amyotrophic lateral sclerosis (ALS) is associated with a significant physical disability. Patients therefore face important and critical choices about palliative care, interventions to support nutrition and respiration and end-of-life issues. As a number of ALS patients bear also cognitive and behavioural impairment, it becomes imperative to understand their ability to properly give consent. Medical decision-making capacity (MDC) is a high-order cognitive capacity relevant for patients, their families and the caring physicians.

**Objective:** We prospectively assessed the MDC in a cohort of non-demented ALS patients using a psychometric instrument and a battery of neuropsychological tests, exploring frontal lobe-related cognitive and behavioural functions.

**Methods:** We enrolled 94 consecutive non-demented ALS patients (n = 94, mean age at onset = 61.8 years + 10, M/F = 1.61). Onset was bulbar in 29 (30.8%) and spinal in 65 (69.2%). All patients underwent the MacArthur Competence Assessment Tool for Treatment (MacCAT-T), a psychometric instrument for assessing decision-making abilities relevant for judgments about patients' competence to consent a treatment. MacCAT-T consists of three main areas of ability, defined as follows: i) understanding (U) the disorder of which the patient is affected and the related treatments; ii) appreciation (A) of the significance of the information; iii) reasoning ® in the process of deciding upon a treatment. For each area, a cut-off value indicating full capacity, marginal capacity or incapacity to consent to a medical treatment was established.

Patients also underwent a comprehensive assessment of the frontal lobe-related cognitive and behavioural functions using, respectively, the phonemic fluencies and both Neuropsychiatric Inventory (NPI) and Frontal System Behavioural Scale (FrSBe).

All data were analysed with ANOVA or, where appropriate, with the rank sum test and $\chi 2$ test. A multivariate analysis was performed to identify the neuropsychological predictors of MDC. Correlations were studied with Spearman rank order test.

**Results:** 27% of the non-demented ALS patients performed poorly during cognitive assessment whereas 46% showed behavioural impairment. However, a high number of patients showed full capacity to consent to a medical treatment (U = 73%; A = 88.3%; R = 76.9%). Site of onset, age at onset, gender, education, FVC, the rate of disease progression did not affect significantly the MDC. The cognitive status was not apparently related to the MDC, whereas the behavioural impairment, as measured by FrSBe, showed a negative impact

of each area (U vs FrSBe: $r = -0.31$, $p = 0.005$; A vs FrSBe: $r = -0.26$, $p = 0.01$, R vs FrSBe: $r = -0.28$, $p = 0.01$).

**Discussion and conclusion:** Most ALS patients show a full capacity to consent to a medical treatment, irrespective of their cognitive impairment. However, those with impaired MDC are also behaviourally impaired. This variable might significantly affect the ability of this group of ALS patients to correctly make medical decisions.

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## C87 LONGITUDINAL COGNITIVE AND BEHAVIORAL SCREENING IN A LARGE US COHORT: RESULTS FROM THE COSMOS STUDY GROUP

WOOLLEY S[1], GOETZ R[2], MURPHY J[3], FACTOR-LITVAK P[2], HUPF J[2], SINGLETON J[2], MITSUMOTO H[2]

[1]*Forbes Norris ALS Research Center, San Francisco, CA, USA,* [2]*Columbia University Medical Center, New York, NY, USA,* [3]*University of California, San Francisco, San Francisco, CA, USA*

*Email address for correspondence: Woolles@sutterhealth.org*

*Keywords: longitudinal, screening, behaviour*

**Background:** Executive dysfunction is a negative prognostic indicator in ALS (1) and cognitive subgroups have different trajectories depending on baseline functioning (2). Behavioural symptoms may predate motor symptoms in ALS (3), yet little is known qualitatively about longitudinal behavioural change.

**Objectives:** To evaluate cognitive and behavioural change in 133 ALS patients screened over 12 months.

**Methods:** Follow-up assessments were completed in 133 ALS patients enrolled in a prospective, interdisciplinary, multicenter, epidemiological study. Measures included: the ALS Cognitive Behavioural Screen (ALS CBS); Written Fluency Index (c words); Frontal Behavioral Inventory-ALS Version (FBI-ALS); Center for Neurological Study-Lability Scale (CNS-LS); ALS FRS-R; FVC. Demographic variables and C9orf status were recorded. Paired t-tests and non-parametric Wilcoxon tests determined change over time. Regression analyses were conducted on cognitive/behavioural variables that exhibited significant change, using baseline scores as the covariate. Post-hoc analyses determined which behavioural items (ALS CBS) or subtests (FBI-ALS) changed significantly over time. A one-way ANCOVA examined change scores on the ALS CBS stratified by baseline cognitive status. Spearman rho correlations examined change scores for ALS-FRS-R subscales and cognitive/behavioural change scores. Significance was set at $p < 0.05$.

**Results**: Clinically significant change in cognition was not detected, regardless of baseline scores. Behavioural scores changed significantly over time (FBI-ALS Disinhibition, $p = 0.005$; FBI-ALS Negative Behaviours, $p = 0.028$; ALS CBS Behaviour Scale, $p < 0.001$). CBS item analysis revealed significant decline in patient frustration tolerance ($p = 0.005$); impaired decision making ($p = 0.007$); reduced adaptability to new situations/changing opinions ($p = 0.008$); decreased emotional responsiveness ($p = 0.017$); increased irritability/anger ($p = 0.039$); altered food preference ($p = 0.049$); decreased insight/denial of problems ($p = 0.013$). Regression analyses indicated that an increase in negative behaviours (ie, apathy) on the FBI scale was associated with increased age ($p = 0.035$). An increase in behavioural problems on the ALS CBS associated with lower ALSFRS-R scores ($p = 0.001$). Pseudobulbar affect (PBA) correlated with the gross motor subscale of the ALS FRS-R ($p = 0.019$) but the presence PBA did not correlate with change in cognition or behaviour. C9orf status did not predict cognitive or behavioural status at 12 months.

**Discussion and conclusion**: Significant behavioural change occurs over a 12-month period. Caregiver-driven screening measures, including the FBI-ALS and ALS CBS, readily detect behavioural change. Behavioural change does not correlate with disease duration or respiratory decline, but may associate with age and functional disability. Controlling for caregiver depressive symptoms may allow future behavioural studies to be strengthened (4).

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## C88 MULTI-DIMENSIONAL APATHY IN AMYOTROPHIC LATERAL SCLEROSIS

RADAKOVIC R[1,2], STARR JM[3], ABRAHAMS S[1,2]

[1]*University of Edinburgh, Edinburgh, UK,* [2]*Anne Rowling Regenerative Neurology Clinic, Edinburgh, UK,* [3]*Alzheimer Scotland Dementia Research Centre, Edinburgh, UK,* [4]*Euan MacDonald Centre for MND Research, Edinburgh, UK*

*Email address for correspondence: radakovic.ratko@gmail.com*

*Keywords: apathy, multidimensional, behavioural assessment*

**Background:** Apathy is a prevalent behavioural symptom of Amyotrophic Lateral Sclerosis (ALS) (1) but assessment is confounded by physical disability. Apathy is thought to be composed of three neurologically distinct subtypes (2) although a comprehensive assessment tool has been lacking. Here we present the new Dimensional Apathy Scale (DAS) that assesses executive, emotional and initiation aspects of apathy and has been specifically designed for neurodegenerative populations with motor impairments (3).

**Objective:** To determine the validity and reliability of the DAS in ALS patients, and their carers, and to explore the substructure of apathy in ALS.

**Methods:** 83 non-demented ALS patients, 75 of their carers and 83 gender-age- education level matched controls were recruited. All participants and their carers completed the DAS, a standard apathy scale- the Apathy Evaluation scale (AES) (4), and the Geriatric Depression Scale-Short form (GDS15) (5, 6).

**Results:** There was a significant dissociation between DAS subscales for both patients and informants, $F(2,296) = 160.30$, $p < 0.001$, with higher scores on the Initiation than the Emotional and Executive subscales.

The patient-control comparison showed a significant interaction effect of Group vs DAS subscale $F(2,328) = 13.86$,

$p < 0.001$, demonstrating that patient and control responses differed between subscales. Post hoc t-tests revealed that Initiation was the only significantly higher subscale, $t$ (64) = 3.22, $p < 0.01$, with patients showing more impairment ($M = 12.5$, $SD = 5.1$) compared to controls ($M = 10.2$, $SD = 4.3$). Additionally, on the Emotional apathy subscale, patients scored ($M = 7.7$, $SD = 3.3$) significantly lower compared to controls ($M = 8.9$, $SD = 3.2$), $t$ (164) = 2.28, $p < 0.05$.

Cronbach's standardized alpha values for DAS subscales were high. The subscales correlated, on average, more positively with the AES than the GDS15, for both patients and informants. There was no significant relationship between the ALS Functional Rating scale and performance on the DAS.

**Discussion and conclusion:** The DAS detects dissociable components of apathy, not confounded by physical disability, and was found to be a reliable instrument with good convergent and discriminant validity for both informant and self-versions. ALS patients showed a specific apathy profile with significantly elevated levels of apathy, resultant from difficulties with initiation of cognition and behaviour, and not emotional or executive apathy components. Future studies will investigate the relationship between apathy dimensions and cognition in ALS and validate the scale in other neurodegenerative disease populations.

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## C89 COGNITIVE IMPAIRMENT AND BEHAVIOURAL CHANGES ARE ASSOCIATED WITH POOR SURVIVAL IN ALS

GOVAARTS R[1], BEELDMAN E[1], RAAPHORST J[1,2], SCHMAND B[1,3], DE HAAN R[4], DE VISSER M[1], VAN DEN BERG LH[1]

[1]*Department of Neurology, University of Amsterdam, Amsterdam, The Netherlands,* [2]*Department of Neurology, Radboud University Medical Centre, Nijmegen, The Netherlands,* [3]*Department of Psychology,* [4]*Clinical Research Unit, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands,* [5]*Brain center Rudolf Magnus Institute of Neuroscience, Department of Neurology, University Medical, Center Utrecht, The Netherlands*

*Email address for correspondence: rosannegovaarts@gmail.com*

*Keywords: cognitive impairment, behavioural changes, survival*

**Background:** Amyotrophic lateral sclerosis (ALS) is relentlessly progressive with a median survival of three to five years. Cognitive impairment and behavioural changes are present in 20–50% of ALS patients. These non-motor changes may negatively influence survival, eg, as a result of refusal of, or reduced adherence to non-invasive ventilation. A robust association between cognitive impairment or behavioural changes and survival has not been established due to correlations of non-motor changes with bulbar onset, the absence of data on ventilation or the use of behavioural scales which have not been validated in ALS in previous studies.

**Objectives:** To examine the association between cognitive impairment and behavioural changes and survival in a cohort of ALS patients, including ALS-FTD patients.

**Methods:** We analysed survival status of ALS patients from two previous cohort studies. Cognitive impairment was defined as a score $< 5^{th}$ percentile on $\geq 2$ tests of executive function, memory or language. Behavioural changes were defined as $\geq 3$ points on $\geq 2$ items on the NeuroPsychiatric Inventory (NPI; n = 22) or $> 22$ points on the ALS-FTD-Questionnaire (n = 108). We performed a Kaplan-Meier survival analysis where survival was defined as time from symptom onset to death. The impact of the explanatory variables (bulbar disease onset, disease severity, age at onset, time to diagnosis and vital capacity) on survival was examined using univariate and multivariate Cox proportional hazards models.

**Results:** One-hundred and thirty-six ALS patients were included in the study (126 ALS; 10 ALS-FTD; 91 men (66.9%), mean age (SD) 61.6 (11.8) years). Median survival time was 4 years and 3 months (95% confidence interval (CI) 3.45–5.05). Eighteen patients (13.2%) had cognitive impairment, 16 patients (11.8%) had behavioural changes and 13 patients (9.6%) had both. Bulbar disease onset was more prevalent among patients with cognitive impairment or behavioural changes (33%) compared to those without (9.5%). Factors associated with shorter survival included bulbar disease onset, disease severity, age at onset, time to diagnosis and vital capacity. In the univariate Cox proportional hazards model, both cognitive impairment and behavioural changes were associated with shorter survival (Hazard ratio (HR) 1.63, 95% CI 1.06–2.51, p = 0.02 and HR 1.97, 95% CI 1.29–3.00, p = 0.002, respectively). Reduced survival was confirmed in a multivariate model for cognitive impairment (adjusted HR 1.82, 95% CI 1.06–3.11, p = 0.03) and behavioural changes (adjusted HR 1.78, 95% CI 1.02–3.12, p = 0.04).

**Discussion and conclusion:** Cognitive impairment and behavioural changes are both associated with reduced survival in ALS patients. This may be partly explained by reduced adherence to non-invasive ventilation, which we are currently investigating in our cohort.

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## C90 PREVALENCE, ASSOCIATIONS AND COURSE OF DEPRESSION IN ALS: OBSERVATIONS FROM A LARGE COHORT

THAKORE N, PIORO E

*Cleveland Clinic, Cleveland, Ohio, USA*

*Email address for correspondence: thakorn@ccf.org*

*Keywords: depression, quality of life, PHQ9*

**Background:** Depression is a treatable complication of ALS that may adversely affect survival.

**Objective:** To study the prevalence, associations and longitudinal course of depression in a cohort of ALS patients.

**Methods:** PHQ9 (a validated depression instrument) and other self-reported measures were prospectively collected from ALS patients on tablet devices prior to their appoint-

ments using software developed in-house (Knowledge Program). Categorical data methods, t-test, logistic regression, and random effects models were used for analysis.

**Results:** Of 931 patients seen over a 7-year period, 825 had at least one PHQ9 recorded and 450 had more than PHQ9 recorded at least 30 days apart. Moderate (PHQ9 $\geq$ 10), moderately severe (PHQ9 $\geq$ 15) and severe depression (PHQ9 $\geq$ 20) were noted initially in 32.8%, 14.8%, and 6.1% of patients, and occurred anytime during the course in 43.4%, 19.6%, and 8.1% of patients respectively; 23.3% of patients were persistently moderately depressed. Lower initial ALSFRS-R (OR 1.08 for each point, CI 1.05–1.11) and pseudobulbar affect (OR 2.29, CI 1.66–3.17) were strongly predictive of depression and remained so in multiple regression models. Other significant predictors on univariate analyses included female gender, older age, predominantly respiratory (but not bulbar) dysfunction, more rapid rate of decline of ALSFRS-R, and lower initial body weight.

PHQ9 was predictive of quality of life (EQ-5D) after controlling for ALSFRS-R, and, additionally, subjective quality of life (EQ-5D VAS) after controlling for EQ-5D valuation. Higher initial PHQ9 and persistent depression were predictive of mortality after controlling for other covariates. Overall, worsening depression was not seen despite motor progression. Patients with worse initial depression often improved, while depression did tend to worsen in patients with rapidly progressive disease.

**Conclusion:** Depression is prevalent in ALS and is strongly associated with disease severity at initial assessment. Paradoxically, however, worsening depression is not observed during follow-up despite disease progression. Depression has detrimental effects on quality of life and survival. ALS patients who are female, older, have pseudobulbar affect, predominant respiratory dysfunction, or lower body weight are more prone to depression and should be screened for early intervention.

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