Introduction

The Motor Neurone Disease Association, in collaboration with the International Alliance of ALS/MND Associations, welcomes you to Brussels for the 25th International Symposium on ALS/MND. This year we are warmly welcomed by our hosts at ALS Liga, Belgium as they celebrate their twentieth anniversary and the symposium reaches its quarter of a century milestone, a huge achievement symbolising 25 years of progress – but still with many questions to be answered.

Many thanks to Professor Wim Robberecht who, after many years of hard work and commitment, chairs the symposium programme committee for the last time this year. Prof Robberecht and the committee have compiled a stimulating and varied platform programme that includes ground breaking scientific topics and thought provoking clinical sessions. Joint opening and closing plenary sessions reflect on the continued need for new treatments and the hurdles that must be overcome in order for a drug to reach clinical trials. Parallel scientific and clinical sessions running for the remainder of the symposium will explore a wide variety of key themes across the field, with a third parallel session focusing specifically on aspects of care practice such as end of life decisions, respiratory management and nutritional assessment.

Once again the quality of the poster presentations is outstanding and with two dedicated poster sessions there will be plenty of time for extensive viewing and lively debates - look out for those posters which have been selected for the poster prize competition. If you are presenting a poster yourself, please upload your final version onto the F1000 poster website to allow even more researchers to access your work, and help us to fulfill our commitment to the international exchange of knowledge. It's a safe and easy way to get your work seen.

Finally, don't forget to use the #alssymp on Twitter to have your say on all symposium related issues; we love to hear your comments and views!

Research Development Team
Motor Neurone Disease Association, Northampton, UK

Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 2014; 15(Suppl. 1): 1–56

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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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.

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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 ± 21 vs 255 ± 20 days). The lifespan was shortened from 381 ± 26 to 339 ± 21 days, representing a decrease of 11%. The disease progression was thus slowed by 75% (48 ± 6.5 vs 84 ± 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 efficac

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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 H2AX6 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.

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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.

Acknowledgements: We would like to thank all the patients and their families who kindly donated tissue for this study.

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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 ± 2.2 days vs. 105.8 ± 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 ALSFRS-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 ALSFRS-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.

Acknowledgements: We thank our patients and their caregivers for the support to our study. We [illegible]

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 Lilla 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 1.23×10^{-9} to 4.75×10^{-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 electro

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 ± 3.4 months (95% CI 36.3 to 49.3) for DPS-3yr, 32.5 ± 1.6 months (29.0 to 41.0) for Lechtzin-3yr, and 31.2 ± 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.

Acknowledgements: The work was supported by the BMBF Joint Project 'MND-NET' as well as the Foundation Georgsmarienhütte and the ALS Initiative 'Aid for People with ALS'.

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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.

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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 ≥ 2 tests of executive function, memory or language. Behavioural changes were defined as ≥ 3 points on ≥ 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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THEME 1 IMPROVING DIAGNOSIS, PROGNOSIS AND DISEASE PROGRESSION

P1 RED FLAGS FOR MUSCLE WEAKNESS: EXPLORING GP DECISION-MAKING AND REFERRAL PATHWAYS

MCDERMOTT CJ[1], BAXTER S[1], BEDFORD J[2]

[1]*University of Sheffield, Sheffield, UK,* [2]*Motor Neurone Disease Association, London, UK*

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

Keywords: referral, diagnosis, referral pathway

Background: Motor neurone disease is heterogeneous in clinical presentation, its clinical course is variable, and several clinical variants are recognized making early referral and diagnosis challenging (1).

Objectives: The data reported here forms part of the evaluation of a checklist for GPs to use with patients that present with muscle weakness. The aim of this phase of the work was to: i) explore GP decision-making processes; ii) to explore factors underpinning referral pathways for patients with muscle weakness.

Methods: The study used qualitative methods to investigate the views and experiences of GPs who have recently referred patients with muscle weakness, and those who have not. Semi-structured interviews were carried out over the telephone, recorded and examined using methods of thematic analysis.

Results: Eighteen GPs were interviewed from a spread of UK regions with a range in level of experience. 13 GPs had referred patients in the last year, and five had not. The most common description of patients who had been referred, or those that were likely to be referred was that of a patient who 'just did not fit' any familiar presentation, and GPs described their feeling of uncertainty, of a pattern that they were unable to explain. The priority was perceived to be referring the patient on to the expert rather than attempting to make a differential diagnosis.

The most commonly described sign that would trigger a referral was the presence of fasciculation. Familiarity with the patient was highlighted as a potentially important element in a GP's ability to assess disease progression. GPs described basing decisions on knowledge mostly acquired during initial training, and identified a range of online resources that they had found helpful. Participants described the challenge of having to make a decision with the patient in front of them, and also the potential impact of mentioning MND. While the majority of referral stories told by participants were of patients being directed rapidly to appropriate pathways, five patients had more convoluted routes to specialist MND services.

Discussion and conclusion: This study highlights the challenges in diagnosis and referral of patients with muscle weakness from primary to specialist care. The presence of fasciculation tended to trigger referral directly to a specialist MND service however patients presenting with other symptoms could experience referral to a range of other agencies. This work provides baseline data prior to the introduction of the Red Flags Checklist by the Royal College of GPs. A second wave of data collection will examine any impact of this tool on decision-making and referral pathways for patients with muscle weakness.

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P2 FOUNDATION OF THE NETHERLANDS ALS CENTRE IN 2003: REDUCING THE DIAGNOSTIC DELAY?

VISSER AE, SEELEN M, VELDINK JH, VAN DEN BERG LH

University Medical Center Utrecht, Utrecht, The Netherlands

Email address for correspondence: a.visser-13@umcutrecht.nl

Background: There is no diagnostic test to confirm the diagnosis of ALS. Due to the relatively low incidence and prevalence of ALS and unfamiliarity with symptoms by general practitioners and other physicians, there is a considerable diagnostic delay.

Objective: We evaluated whether the founding of the Netherlands ALS centre in 2003, consisting of the University Medical Centre Utrecht and the Amsterdam Medical Centre, and a national awareness campaign resulted in the reduction of the diagnostic delay.

Methods: The Prospective ALS study the Netherlands database provided data such as the date and site of onset, the date and hospital of diagnosis, El Escorial Criteria (EEC) and additional clinical characteristics of patients with ALS and PMA. The diagnostic delay was calculated in months and Chi^2 tests were performed. Hypotheses on site of onset and EEC influencing the diagnostic delay were analysed.

Results: Out of 2350 patients, we found a reduction in mean diagnostic delay of 1.1 months when comparing diagnostic period 2003–2007 with 2008–2012. In addition, within these periods, more patients were diagnosed within 12 months after symptom onset ($p < 0.001$). Finally, specifically within the group of patients with less clinical certainty (probable - laboratory supported and possible EEC) and in the group of patients with a spinal site of onset, more patients were diagnosed within

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12 months after symptom onset between 2008–2012 compared to 2003–2007 ($p = 0.002$ and $p = 0.004$ respectively).

Discussion and conclusion: After the foundation of the ALS centre in 2003, more patients were diagnosed within 12 months. This is the result of the expanding expertise and well-organised diagnostic workup, especially in those patients where there is less clinical certainty about the diagnosis. A shorter diagnostic delay leads to earlier treatment with Riluzole, and provides a greater opportunity to become enrolled in clinical trials and for the appropriate planning of care.

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P3 EXTENDING THE PHENOTYPE OF FOSMN SYNDROME: AN IMPORTANT ALS MIMIC

BROAD R, LEIGH PN

Brighton and Sussex Medical School, Falmer, East Sussex, BN1 9RY, UK

Email address for correspondence: r.broad@bsms.ac.uk

Keywords: FOSMN, blink reflex, trigeminal nuclei

Background: Facial Onset Sensory Motor Neuronopathy (FOSMN) is a rare disorder (1) presenting with facial numbness, facial weakness, and bulbar symptoms and progressing to involve the neck, shoulder girdle, and limb muscles (1–5). The pathogenesis of FOSMN remains unknown, however, evidence supports a neurodegenerative process with early involvement of the trigeminal nuclei (2–4). A link between FOSMN and amyotrophic lateral sclerosis (ALS) has been proposed (2,4,5) and as the number of reported cases increases, the phenotypic spectrum of FOSMN continues to expand (1–5).

Objectives: To study the phenotypic heterogeneity in FOSMN and to highlight a case of motor onset FOSMN masquerading as an ALS mimic.

Methods: Six patients with FOSMN were identified following thorough clinical assessment and investigation.

Results: All cases were male, aged between 42 and 66 years. Five presented with features of central trigeminal sensory dysfunction. All patients had facial and neck weakness. Four had additional limb weakness. Three patients suffered with severe neck pain. Creatine Kinase was mildly elevated in two patients. Blink reflexes were delayed or absent. Disease duration ranged from 18 months to over ten years.

In one case, FOSMN was altered with a predominantly motor syndrome presentation.

Case study: In this incident, a 55 year-old man presented with a decade of progressive facial and arm wasting and developed significant neck pain. The patient experienced mild difficulty swallowing and had lost over a stone in weight. Examination revealed wasting and mild weakness of facial, neck, shoulder girdle, and proximal arm muscles. Tendon reflexes were brisk at the knees with bilateral adductor jerks. EMG studies showed chronic partial denervation of the upper limbs and the cervical paraspinal muscles with some fasciculation. Blink reflexes showed bilaterally delayed responses.

Discussion and conclusion: FOSMN may appear as an atypical case of ALS, particularly when features are predominantly motor and there is upper motor neurone involvement. TDP-43 inclusions have been demonstrated in one case of FOSMN (4) and heterozygous D90A SOD 1 mutation was reported in another case of FOSMN (5) supporting a link FOSMN and ALS. However, FOSMN can usually be distinguished from ALS. Neck pain is a striking feature and blink reflex abnormality is diagnostically useful (1–5).

The case described in this study, expands the phenotype of FOSMN to include a predominantly motor syndrome and highlights the importance of considering FOSMN as an ALS mimic.

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P4 SPORADIC LOWER MOTOR NEURON DISEASE WITH A SNAKE EYES APPEARANCE ON THE CERVICAL ANTERIOR HORNS BY MRI: A NEW CLINICAL SUBTYPE?

SASAKI S

Tokyo Women's Medical University, Tokyo, Japan

Email address for correspondence: ssasaki@nij.twmu.ac.jp

Keywords: lower motor neuron disease (LMND), snake eyes appearance, new clinical subtype

Background: Lower motor neuron syndromes are characterized by progressive, asymmetrical lower motor neuron symptoms and largely classified into two subgroups: one in which motor neurons are primarily affected (lower motor neuron diseases: LMNDs), and the other, in which motor axons and their surrounding myelin are predominantly affected, leading to muscle weakness and atrophy, fasciculation and muscle cramps. These syndromes include various diseases. Thus, 'LMND' is the term generally used to describe diseases in which only LMN signs are detected.

Objectives: To elucidate whether certain patients with slowly progressive, asymmetric, pure lower motor neuron upper limb weakness showing a snake eyes appearance by MRI constitute a new clinical subtype of LMND.

Methods: For more than 5 years, the author regularly followed up two unique unprecedented LMND patients with a longstanding clinical course of more than 10 and 7 years (aged 52 and 40 years at onset), who presented with proximal dominant and distal dominant spinal muscular atrophy localized in the upper extremities, respectively, with unilateral predominance and a snake eyes appearance by MRI.

Results: Patients were characterized by 1) longstanding slow progression or stability of lower motor neuron signs over a long period of time localized exclusively in the upper extremities with unilateral predominance and distal or proximal preponderance; 2) the absence of upper motor neuron signs, bulbar signs, sensory disturbances and respiratory involvement; 3) a snake eyes appearance on the anterior horns of the cervical cord by axial T2-weighted MRI; 4) neurogenic change with denervation potentials such as fasciculation confined to the affected muscles by EMG; 5) a normal creatine kinase

level. Patients neither fall into any existing category of LMND such as progressive muscular atrophy or flail arm syndrome, nor indicate other lower motor neuron syndromes with or without a snake eyes appearance.

Discussion and conclusion: These two unique unprecedented LMND patients with a longstanding clinical course localized exclusively in the upper extremities showing a snake eyes appearance on the anterior horns of the cervical cord by axial T2-weighted MRI may constitute a new clinical subtype of LMND.

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P5 AMYOTROPHIC LATERAL SCLEROSIS (ALS) WITH LABORATORY ABNORMALITIES OF UNKNOWN SIGNIFICANCE (LAUS)] – WHERE DOES IT BEGIN AND WHERE DOES IT END?

BROOKS BR[4], BRAVVER EK[1,4], DESAI UG[1,4], STORY SJ[1,4], LINDBLOM SS[2,4], PACICCO TJ[2,4], BOCKENEK WL[3,4], SANJAK MS[1,5], WARD AL[1,6], WRIGHT KA[1], LANGFORD VL[1], HOLSTEN SE[1], FISCHER MP[1], LARY C[1], NEMETH J[1], BELCHER SL[1], NICHOLS MS[1], SMITH NP[1], LUCAS NM[1], OPLINGER H[1]

[1]*Carolinas Neuromuscular/ALS-MDA Center - Carolinas Medical Center - Department of Neurology – Carolinas Healthcare System Neurosciences Institute, Charlotte, North Carolina, USA,* [2]*Department of Internal Medicine - Carolinas Medical Center, Charlotte, North Carolina, USA,* [3]*Department of Physical Medicine and Rehabilitation - Carolinas Rehabilitation, Charlotte, North Carolina, USA,* [4]*University of North Carolina School of Medicine – Charlotte Campus, Charlotte, North Carolina, USA,* [5]*Department of Kinesiology, University of North Carolina - Charlotte, Charlotte, North Carolina, USA,* [6]*Cabbarus College of Health Sciences - Occupational Therapy, Concord, North Carolina, USA*

Email address for correspondence: benjamin.brooks@carolinashealthcare.org

Keywords: ALS-LAUS, MGUS, anti-ganglioside antibody

Background: Amyotrophic lateral sclerosis (ALS) with laboratory abnormalities of unknown significance (ALS-LAUS), characterized by upper and lower motor neuron signs together with LAUS, has been categorized as a diagnostic classification category by the World Federation of Neurology Research Group on Motor Neuron Disease/ALS (1). Lower motor neuron syndromes (LMNS) with LAUS may be separated out as ALS-Mimics (2,3).

Objectives: To define the prevalence/natural history of ALS-LAUS at an ALS Multidisciplinary Clinic in the Southeastern USA.

Methods: Patient Database review from 2010–2014 of 457 patients referred for ALS diagnosis.

Results: ALS-LAUS was seen in 17.2 % (79/457) of evaluated patients with probable-laboratory supported, clinically probable and clinically definite ALS by Revised El Escorial criteria (1). GM1 ganglioside/HS6S/SGPG Antibodies (Abs) (12/79 = 15.2%); Monoclonal Gammopathy of Unknown Signficance (MGUS)/Waldenstrom (16/79 = 20.3%); Voltage-Gated Calcium or Potassium Channel Abs (7/79 = 8.8%); hypo/hyper-gammaglobulinemia/cryoglobulinemia (12/79 = 15.2%); Acetylcholine Receptor/Ganglionic Acetylcholine Receptor/Skeletal muscle Abs (3/79 = 3.8%); Anti-phospholipid Abs (2/79 = 2.5%) comprise a pattern of possibly immune-mediated motor neuron pathogenesis (52/79 = 65.8%). Concurrent infection with virus (WNV, HCV, HPV, Rubella, 5/79 = 6.3%) and borrelia burgdorferi (1/79 = 1.3%) was also identified. Methylmalonic academia (4/79 = 5.1%) and aluminum toxicity (4/79 = 5.1%) were identified and treated.

In addition to standard Riluzole, patients with ganglioside Abs (10/12 = 83.3%), MGUS/Waldenstrom (2/16 = 12.5%); VGCC/KC Abs (4/7 = 57.1%) or hyper-or hypo-gammaglobulinemia/cryoglobulinemia (1/12 = 4.8%) were treated with IVIg and/or other regimens. On IVIg treatment, ALSFRS-R deterioration showed no change over 12 months in 1/2 MGUS/Waldenstrom patients and slowed in 3/10 ganglioside Abs patients. Pulmonary embolism rate (5/79 = 6.3%-ALS-LAUS; 20/377 = 5.3%-ALS) was comparable in both groups.

Discussion and conclusion: Further detailed analysis of progression rate by site of onset, sex, age, treatment will require the assimilation of clinic-based datasets of properly analysed ALS-LAUS patients from multiple clinic sites. The appropriate role of IVIg in ALS-LAUS patients requires further study following clarification of the natural history of these patients compared with non-ALS-LAUS patients. The determination as to whether auto-antibodies to additional antigens may play a role in the progression rate of ALS-LAUS compared with sporadic ALS needs to be systematically studied (4,5).

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P6 PARANEOPLASTIC SUBACUTE LOWER MOTOR NEURON SYNDROME ASSOCIATED WITH SOLID CANCER

VERSCHUEREN A[1], GALLARD J[1], ATTARIAN S[1], BOUCRAUT J[3], HONNORAT J[2], POUGET J[1]

[1]*Reference centre for neuromuscular diseases and ALS, Hôpital de la Timone, marseille, France,* [2]*French Reference Center on Paraneoplastic Neurological Syndrome, Hospices Civils de Lyon, lyon, France,* [3]*Laboratoire d'immunologie, Hôpital de la Conception, marseille, France*

Email address for correspondence: annie.verschueren@ap-hm.fr

Keywords: LMND, paraneoplastic, solid cancer

Objectives: To describe paraneoplastic lower motor neuron syndrome associated with solid cancer and/or anti-neuronal antibodies.

Methods: We retrospectively analysed three patients with pure lower motor neuron syndrome and who were followed for more than four years in our center. The investigations led to the diagnosis of paraneoplastic syndrome.

Results: The three patients shared several common clinical features, including a rapidly progressive lower motor neuron syndrome over the course of a few weeks leading to a severe functional impairment. The neurological symptoms preceded the diagnosis of a breast adenocarcinoma and a thymoma in the first two patients with anti-beta IV spectrin and anti-CV2/CRMP5 antibodies, respectively. Cancer was not detected in the third patient who had circulating anti-Hu antibodies. Electrodiagnostic studies revealed pure motor axonal involvement, abundant fasciculations, and acute denervation restricted to the lumbosacral, cervicothoracic, or both spinal segments, consistent with subacute lower motor neuron syndrome. Cerebral spinal fluid analysis showed an intrathecal synthesis of immunoglobulins in two out of three patients, but was otherwise normal. A final diagnosis of paraneoplastic syndrome was made after investigations for alternative causes of lower motor neuron syndrome. Early diagnosis, combined treatment of the underlying cancer, and immunomodulatory treatment, led to neurological improvement of the disease in two out of the three cases in which the cancer was diagnosed. The third patient, without cancer, died within a few months after the onset of neurological symptoms.

Discussion and conclusion: Cases of subacute lower motor neuron syndrome with rapid progression may occur as an expression of a paraneoplastic neurological syndrome, and an active research of cancer is justified in these cases. Anti-neuronal antibodies and CSF analysis can help to identify the autoimmune nature of the disease. Identification of these syndromes is important, as the treatment of underlying malignancy along with immunomodulatory treatment may result in a favourable long-term outcome of these potentially fatal diseases.

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P7 EXTRAPYRAMIDAL SYNDROME IN SPORADIC UPPER MOTOR NEURON-DOMINANT ALS WITH PURE TDP-43 PATHOLOGY

PRUDLO J[1], KASPER E[2], SCHUSTER C[3], KRAUSE BJ[4], BISKUP S[5], WALTER U[6], BENECKE R[6], BÜTTNER A[7], BESCHORNER R[8], NEUMANN M[8]

[1]*Department of Neurology, University of Rostock and DZNE, Rostock, Germany,* [2]*Department of Psychosomatic Medicine, University of Rostock, Rostock, Germany,* [3]*Academic Unit of Neurology, Trinity College, Dublin, Ireland,* [4]*Department of Nuclear Medicine, University of Rostock, Rostock, Germany,* [5]*Department of Neurodegenerative Diseases, Hertie Institute for Clinical Brain Research, Tuebingen, Germany,* [6]*Department of Neurology,* [7]*Institute of Forensic Medicine, University of Rostock, Rostock, Germany,* [8]*Department of Neuropathology, University of Tuebingen and DZNE, Tuebingen, Germany*

Email address for correspondence: johannes.prudlo@med.uni-rostock.de

Keywords: extrapyramidal, UMN-dominant, TDP-pathology

Background: In amyotrophic lateral sclerosis (ALS), the cerebral TPD-43 pathology is spread beyond the pyramidal motor system. As regards the basal ganglia, this pathology is predominantly subclinical. However, the upper motor neuron (UMN)-dominant ALS variants, in particular, can clinically be associated with extrapyramidal features, suggesting overlap syndromes and distinct pathologies.

Objectives: To correlate clinical and pathological data in an unusual sporadic UMN-dominant ALS case with prominent extrapyramidal abnormalities.

Case Report: A 58-year-old male had difficulties walking and dysarthria, later experiencing numerous falls before becoming wheelchair-bound within 16 months. He showed bulbar-spinal spastic quadriparesis with slight extraocular and sphincter involvement; additionally, he demonstrated non-drug-induced bradykinesia, hand athetosis (swan-neck deformity), and foot and facial dystonia, all of which without a response to levodopa. He died three years after the disease onset. In the second year of this period, he developed lower motor neuron signs, thus fulfilling the criteria of definite ALS. Shortly before his death, he became mute, suffered from severe dysphagia (asphyxia by alimentary bolus) and displayed a marked pseudobulbar affect, behavioural changes (disinhibition), and restlessness. His cognition remained unaffected. The FTD criteria were not fulfilled.

Results: The dopamin transporter SPECT (DaTSCAN) showed an asymmetric reduction in striatal tracer uptake. Transcranial ultrasound showed a marked hyperechogenenicity of the substantia nigra. No mutations were detected in the ALS associated genes: SOD-1, Alsin, *C9orf72*, TARDBP, or FUS. At autopsy, degeneration of the pyramidal motor system (UMN$>$LMN) and a moderate degeneration of the substantia nigra pars compacta was observed. Immunohistochemistry revealed TDP-43 positive inclusions in neurons and oligodendroglial cells in these brain regions and also in the putamen and pallidum, while extramotor cortex (frontal, temporal, parietal) and hippocampus were devoid of TDP-43 pathology. No protein deposits characteristic for other neurodegenerative diseases such as Lewy bodies, neurofibrillary tangles or senile plaques were observed by immunohistochemistry for tau, alpha-synuclein, and b-amyloid.

Discussion and conclusion: The ALS-Plus syndrome presented here is not based on the coincidence of two independent neurodegenerative diseases. The striking extrapyramidal abnormalities were caused instead by pure TDP-43 pathology and correlated with the distribution and pronounced severity of the TDP-43 pathology in the basal ganglia and midbrain in this patient compared to that usually observed in cognitively normal ALS-patients. Our case is a further example of the clinical heterogeneity of pure TDP-43 proteinopathies (1).

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P8 MAGNETOENCEPHALOGRAPHIC EVIDENCE OF CORTICAL MOTOR DYSFUNCTION IN ALS

PROUDFOOT M[1], ROHENKOHL G[1], GOULD I[1], TALBOT K[1], WOOLRICH M[1], NOBRE AC[1], WUU J[2], BENATAR M[2], TURNER M[1]

[1]*University of Oxford, Oxford, UK,* [2]*University of Miami, Florida, USA*

Email address for correspondence: malcolm.proudfoot@stx.ox.ac.uk

Keywords: magnetoencephalography (MEG), neurophysiology, biomarkers

Background: Neurophysiological studies in ALS have supported evidence of cortical motor network dysfunction, most notably the phenomenon of hyperexcitability. The temporal sensitivity of magnetoencephalography (MEG) enables selective analysis of a brief period of motor preparation. Motor network activity is therefore measurable regardless of subsequent action completion, overcoming a key limitation of task-based functional MRI. The distortion-free MEG signal also permits more confident anatomical localization of oscillatory dysfunction.

Objectives: To pilot MEG in a study of affected ALS patients, including pre-symptomatic individuals at high genetic risk, with the goal of developing novel neurophysiological biomarkers that might improve patient stratification and ultimately would be relevant to future studies of disease prevention.

Methods: Eleven affected patients (8 ALS, 3 PLS; mean age 63.0 ± 7.8), 11 pre-symptomatic mutation carriers (10 SOD1, 1 *C9orf72*, mean age 52.2 ± 10.2) and 10 healthy controls (mean age 57.5 ± 11.6) were studied. Action preparation and completion was studied during MEG acquisition using a simple cued go/no-go task requiring manual responses with either index finger. A central visual cue incorporated both spatial and temporal information for the participant, indicating whether to use the left or right hand for the response, and whether to expect a one or two second interval from the cue to the go-no-go instruction. Data were analysed using a locally developed toolset based upon similar principles to functional MRI analysis, including independent component analysis artefact identification, established 'beamformer' reconstruction of whole brain cortical MEG sources, general linear model analysis of experimental and group contrasts, and threshold cluster statistics.

Results: The MND group had slower reaction times compared to controls (mean 525ms versus 453ms, $p < 0.001$), while the pre-symptomatic mutation carriers responded faster but also made more no-go errors compared to controls (mean reaction time 399ms versus 453ms; errors 26.2% versus 17.9%, $p < 0.001$). In all 3 groups, MEG time frequency analysis revealed functional cortical oscillatory activity, most notably a lateralized beta-band desynchronization during movement preparation, and rebound synchronization after movement termination. Group comparisons revealed delayed contralateral beta-band desynchronization and rebound. Source reconstruction to a standard MRI template mapped these abnormalities to the precentral gyrus. Qualitatively similar, but quantitatively smaller effects were seen in the pre-symptomatic group.

Discussion and conclusion: These pilot findings suggest that beta-band activity is a candidate MEG-based biomarker for motor system dysfunction in ALS. It may shed light on both pathological and compensatory cortical processes, including those occurring before the development of symptoms. Functional neurophysiology might also be expected to respond more readily to pharmacological intervention and, with continued development; MEG will merit consideration as a source of pharmacodynamic biomarkers for CNS drug activity.

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P9 PROGNOSTIC FACTORS ON THE COURSE OF FUNCTIONAL STATUS OF PATIENTS WITH ALS; A SYSTEMATIC REVIEW

CREEMERS H[1], GRUPSTRA H[1], NOLLET F[1], VAN DEN BERG LH[2], BEELEN A[1]

[1]*Department of Rehabilitation, Academic Medical Center, Netherlands ALS Center, Amsterdam, The Netherlands,* [2]*Department of Neurology, Rudolf Magnus Institute of Neuroscience, Netherlands ALS Center, Utrecht, The Netherlands*

Email address for correspondence: h.w.creemers@amc.uva.nl

Keywords: patient counselling, prognosis, disease progression

Background: The progressive course of ALS results in a broad and ever-changing spectrum of the care needs of patients with ALS. The timing of appropriate interventions requires accurate prediction of the individual course of the disease. In clinical practice individual prognosis is largely based on the clinician's cumulated experience. Clinical prognostic tools for the functional course are lacking. Knowledge of prognostic factors for the functional course of ALS may enhance clinical prediction.

Objectives: To review the evidence regarding prognostic factors for the rate of decline in functional status of patients with ALS.

Methods: We searched the electronic databases Medline, EMBASE, CINAHL, PsycINFO, and Web of Science for longitudinal cohort studies reporting on prognostic factors for the course of the functional status, assessed with versions of the ALS Functional Rating Scale.

Two reviewers independently assessed the methodological quality of the included studies using the QUIPS tool. The overall quality of evidence for each prognostic factor was assessed using the GRADE approach, considering risk of bias, imprecision, inconsistency, in directness and publication bias.

Results: We included 9 prospective and 4 retrospective cohort studies with sample sizes ranging from 31 to 2452 patients, examining a wide variety of prognostic factors for a decline in ALSFRS(-R) total score (13 studies) or domain scores (one study). Five studies included an inception cohort, one study presented data of a population based ALS register. Thirteen potential prognostic factors for a decline in ALSFRS(-R) total score were studied in more than one cohort study. Based on the GRADE approach, the quality of evidence for the prognostic value of age at onset, site of onset, time from symptom onset to diagnosis, and ALSFRS-R baseline score was low, mainly due to the limited data and inconsistency of results in the small number of studies included. The prognostic value of age at diagnosis, forced vital capacity, frontotemporal dementia, body mass index and comorbidity remains unclear due to the limited number of studies and small sample sizes.

Discussion and conclusion: The low level of evidence for factors that are predictive for a decline in ALSFRS(-R) may

be due to the lack of unidimensionality of the scale and the heterogeneity of the ALS syndrome. Progress in understanding the genetics of the disease is needed to identify subtypes of ALS that allow for better prognosis.

The current evidence on prognostic factors for functional decline in ALS is insufficient to allow the development of a prediction tool that can support clinical decisions. Given the limited data, future prognostic studies may need to focus on factors that have a predictive value for a decline in ALSFRS(-R) domain scores, preferably based on internationally collected and shared data.

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P10 HYPONATREMIA IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS: AN INDICATOR OF RESPIRATORY FAILURE?

ABSTRACT WITHDRAWN

P11 ALS ONSET AND PROPAGATION: INSIGHT FROM RESPIRATORY FUNCTION TEST

SOHN S-Y[2], KIM D-G[1], HONG Y-H[3], PARK K-H[2], SUNG J-J[2], LEE K-W[2], PARK K-S[1]

[1]*Seoul National University Bundang hospital, Seongnam, Gyeonggi-do, Republic of Korea,* [2]*Seoul National University Hospital, Seoul, Republic of Korea,* [3]*Boramae Hospital, Seoul, Republic of Korea*

Email address for correspondence: chungmoon@hanmail.net

Keywords: respiratory function test, respiratory muscle strength test, disease progression

Background: Amyotrophic lateral sclerosis (ALS) develops clinical manifestations at a focal body and spreads to other regions. There is controversy surrounding the onset of neuronal change (focal, multifocal or ubiquitous) and propagation mechanism (contiguous or non-contiguous). Maximal inspiratory pressure (MIP) reflects the strength of the diaphragm, main inspiratory muscle, and other inspiratory muscles that are innervated by cervical spinal roots (C3, C4, C5) and thoracic roots (T1–12). Maximal expiratory pressure reflects the strength of expiratory muscles which is innervated by thoracic roots (T1–12).

Objectives: We hypothesized that the MIP and MEP ratio will be bigger in caudal region onset patients compared to rostral region onset patients, at the early stage of ALS, without severe respiratory dysfunction according to the focal onset, contiguous propagation theory. We performed a retrospective study to compare parameters of respiratory muscle strength tests between ALS patients with different regions of onset.

Methods: A retrospective study was performed in 114 ALS patients who clinically and electro-physiologically fulfill El Escorial criteria of definite and probable ALS. Patients carried out a battery of respiratory tests: spirometry, forced vital capacity (FVC), forced expiratory volume in one second (FEV1), MIP, MEP and stiff nasal inspiratory pressure (SNIP). MIP, MEP, SNIP, MIP per MEP ratio and SNIP per MEP ratio was compared between the patients with different regions of onset. The muscle strength and spirometry profile among patients who did not show severe respiratory dysfunction (FVC > 50%) was also determined.

Results: In this study 25 bulbar onset (male 14, female 11), 65 cervical onset (male 42, female 23), and 23 lumbosacral onset (male 17, female 6) ALS patients were analysed. The mean time from symptom onset to clinical examination was 20.6 months (2–74 months). 74 patients showed FVC > 50%: 14pts from the bulbar onset group (14/26), 40 from cervical region onset group (40/65), 20 from lumbosacral region onset group (20/23) ($p = 0.013$).

We compared respiratory muscle strength and spirometry profiles among patients with FVC > 50%. There was no difference in FVC between groups with FVC over 50% ($p = 0.22$). MIP and MIP/MEP ratio was lower in bulbar onset patients compared with cervical and lumbosacral onset patients ($p = 0.01$, $p = 0.017$). But SNIP and SNIP/MEP ratio showed no difference between groups ($p = 0.742$, $p = 0.292$).

Discussion and conclusion: There is a difference in FVC between ALS patients with different regions of onset. MIP and MIP/MEP were lower in bulbar onset compared with limb onset ALS. MIP might be influenced with bulbar dysfunction because there was no difference in MEP, SNIP, and SNIP/MEP ratio between ALS patients. These clinical findings cannot be explained by focal onset and propagation hypothesis.

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P12 THE RELATIONSHIP BETWEEN VOLUNTARY COUGH PRODUCTION AND SWALLOW SAFETY IN INDIVIDUALS WITH AMYOTROPHIC LATERAL SCLEROSIS

PLOWMAN EK, WATTS S, DOMER A, GAZIANO J, TABOR L

University of South Florida, Tampa, Florida, USA

Email address for correspondence: plowman@usf.edu

Keywords: aspiration, cough, dysphagia

Background: Cough is an essential airway protective mechanism and is particularly important for those with disordered swallowing. With the necessity for fine-tuned laryngeal and respiratory coordination for both cough and swallow, we hypothesize that dystussia (disorder of cough) may be predictive of swallowing dysfunction in individuals with Amyotrophic Lateral Sclerosis (ALS).

Objectives: To examine the relationship between voluntary cough productions and swallow safety in individuals with ALS.

Methods: 27 patients with ALS (El-Escorial criteria) underwent a standardized video fluoroscopic evaluation of swallowing and completed voluntary cough spirometry testing. Physiologic measures of voluntary cough production from 16 individuals with ALS showing no video fluoroscopic evidence of penetration/aspiration were examined and compared to 11 ALS participants with evidence of penetration/aspiration. Group differences were assessed using a one-way ANOVA and a series of Spearman's Rho correlations performed to assess the degree of relationship between voluntary cough measures and airway safety during swallowing. Alpha was set at 0.05.

Discussion and conclusion: The penetrator/aspirator group presented with significantly lower cough volume acceleration ($F(1,26) = 4.77$, $p < 0.05$). Mean cough volume acceleration was 76.38 (SEM = 13.59) for ALS non-penetrator/aspirators

vs. 40.01 (SEM = 6.62) for ALS penetrator/aspirators. In addition, a significant negative correlation was revealed between PAS score and cough volume acceleration ($r = 0.48$, $p < 0.05$) indicating that the higher degree of airway invasion of material during swallowing (PAS score), the lower the cough volume acceleration observed. Compression phase duration of voluntary cough was significantly longer for ALS patients who penetrated/aspirated, ($F(1,26) = 3.93$, $p < 0.05$) indicating increased time to glottic closure during voluntary cough production.

In this study, a relationship was observed between voluntary cough production and airway safety/protection during swallowing. ALS patients who penetrated/aspirated demonstrated weaker and less efficient voluntary cough production as evidenced by lower cough volume acceleration and increased time to glottic closure during cough (longer compression phase durations). Measures of voluntary cough may be useful predictors of penetration and aspiration in individuals with ALS. Voluntary cough could prove a useful screening tool to aide in the evaluation of airway protection in individuals with ALS, however further investigation is warranted to validate these initial findings.

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P13 CLINICAL CORRELATION OF THE YAWNING REFLEX IN ALS PATIENTS

KWAN J, DIAZ M, ALTEMEMI N, ZILLIOX L, EMPENO R, RUSSELL J

University of Maryland, Baltimore, MD, USA

Email address for correspondence: jkwan@som.umaryland.edu

Keywords: yawn, sleep disorder, upper motor neuron dysfunction

Background: Severe and excessive yawning is a symptom that has been observed and described in ALS patients (1, 2). Frequent yawning can also be present in several neurological disorders but the cause for this symptom is poorly understood (3). In ALS, patients who have bulbar onset symptoms, more frequently report excessive yawning (2), in addition, the yawning reflex has been suggested to be a sign of upper motor neuron dysfunction (4).

Objectives: The aim of this study is to determine the frequency of excessive yawning in ALS patients and to examine the clinical correlate for this symptom.

Methods: A prospective study was conducted to determine the frequency of yawning in consecutive ALS patients evaluated at University of Maryland ALS Clinic. A patient self-reported yawning scale was administered and the severity of yawning was determine as none/normal, moderately excessive, and severely excessive. Additional clinical parameters collected from each patient include: date of symptom onset; date of diagnosis; site of symptom onset; clinical rating of cortical motor neuron dysfunction; ALSFRS-R; forced vital capacity; arterial blood gas (in patients whose FVC was less than 70% of predicted); Epworth Sleepiness Scale; CNS lability scale for pseudobulbar affect; and ALS depression inventory scale.

Results: 30 consecutive ALS patients were evaluated for this study. Four patients reported severely excessive yawning, nine patients reported moderately excessive yawning, and 16 patients reported no increase in yawning. Excessive yawning was significantly correlated to excessive sleepiness as determined by the Epworth Sleepiness Scale. ALS patients who had excessive yawning were more likely to have excessive sleepiness. In patients with excessive yawning, there was a trend towards greater clinical findings of upper motor neuron impairment, shorter duration until onset of bulbar symptoms, and shorter disease duration. There was no association between excessive yawning and respiratory parameters or severity of bulbar dysfunction.

Discussion and conclusion: Excessive yawning is a frequent symptom in ALS patients. In this study, excessive yawning is associated with excessive daytime sleepiness.

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P14 AMYOTROPHIC LATERAL SCLEROSIS AND OXIDATIVE STRESS BIOMARKERS IN RELATION TO PHYSICAL EXERCISE

CHICO L, LOGERFO A, CALDARAZZO EI, PETROZZI L, ROCCHI A, CARLESI C, ORSUCCI D, SCHIRINZI E, SIMONCINI C, MANCUSO M, SICILIANO G

University of Pisa, Pisa, Toscana, Italy

Email address for correspondence: lucia.chico@katamail.com

Keywords: oxidative stress, biomarkers, exercise

Background: Amyotrophic lateral sclerosis (ALS) is a progressive neuromuscular disease for which there is no cure. Among the assumptions made regarding the pathogenesis of ALS, the most reliable, call into question the oxidative stress, as well as the excitotoxic theory and alterations of mitochondria (1).

Oxidative stress describes a condition in which the antioxidant defences are unable to maintain cellular ROS levels below the toxicity threshold. This could be the result of an excessive ROS production, or a loss of the natural antioxidant defences, or of both factors. There is a general misunderstanding among healthcare professionals of the proper use and potential benefits of physical therapy to treat the symptoms and resulting loss of independence. Physical activity leads to a temporary imbalance between the ROS production and their disposal and, therefore, could be the primary cause of oxidative stress, however, there are currently major limitations of the knowledge of the relationship between oxidative stress and performance (2).

Objectives: To investigate, through the monitoring of certain biological markers, some alterations of the mechanisms that underlie the regulation of cellular response against oxidative stress in relation to the exercise, in order to verify a possible correlation between exercise and increases in these parameters.

Methods: The work was divided into two experimental phases: in the first phase markers of oxidative damage in 32 ALS patients (mean age 63.6 ± 10.8) at diagnosis and in 54 healthy volunteers (mean age 69.9 ± 9.2) were measured in order to check a possible alteration of the cellular state redox in patients compared to controls. Following discharge, 11

patients have conducted, for a period of 50 days, an aerobic workout of moderate intensity, while the remaining 21 have not been subjected to any training program.

Results: The results obtained, confirm the redox alteration in ALS patients with sporadic form of diagnosis. What emerged from the comparison of these parameters before and after the training period is that these values have remained constant over time, while there has been an increase of oxidative damage in patients who received no training ($p < 0.01$).

Discussion and conclusion: Despite the aggressive and rapid progression of the disease, a moderate-intensity exercise may be helpful to maintaining the welfare of the musculoskeletal system.

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P15 SIT TO STAND (STS) RATING SCALE: CONSTRUCT VALIDITY OF A NOVEL MEASURE OF LOWER EXTREMITY (LE) FUNCTION IN ALS PATIENTS

SANJAK MS[1,2], OLDHAM J[2], BOYLES G[1], HOLSTEN SE[1,3], BOCKENEK W[1,3], STORY SJ[1,3], LINDBLOM SS[1,3], PACCICO T[1,3], BRAVVER E[1], RUSSO PC[1], BROOKS BR[1]

[1]*Carolinas Healthcare System, Carolinas Neuromuscular, ALS-MDA Center, Charlotte, NC, USA,* [2]*Department of Kinesiology, University of North Carolina, Charlotte, NC, USA,* [3]*Univ North Carolina Sch of Med, Charlotte, NC, USA*

Email address for correspondence: Mohammed.Sanjak@carolinashealthcare.org

Keywords: sit to stand (STS), lower extremity strength, mobility

Background: The STS manoeuvre is essential to everyday physical function and independent living. Loss of STS ability can be devastating for patients' quality of life, as it precedes loss of independence in other fundamental motor functions such as transfer and ambulation. LE muscle strength and power are the main determinants of the STS manoeuvre. A standardized, valid, quick, and easy-to-perform descriptive rating scale that reflects the clinical level of impairment of LE function in ALS patients is lacking.

Objectives: To establish a novel 5 point rating scale for the STS manoeuvre in individuals with ALS.

Methods: A five point scale ranging from 0–4 was developed to reflect patients' level of impairment during the STS manoeuvre. The scale was based on the level of assistance needed to complete the manoeuvre, and was graded as follows: 4 = no arm or compensation; 3 = use of one arm; 2 = use of two arms; 1 = minimal/moderate assistance of one person; 0 = cannot stand/requires lifting. The STS evaluation was performed on 69 individuals with ALS. Bilateral LE muscle strength was evaluated by measuring maximum voluntary isometric contractions (MVIC) of ankle dorsiflexors, knee extensors and flexors, and hip extensors and flexors using computerized fixed dynamometry. In addition, walking capacity was evaluated by the 6 minute walk test (6MW), gait speed by the 25 foot walk test (25 FWT), and mobility and balance by the timed up and go (TUG) test. Pearson correlation coefficients were calculated to identify linear relationships between the STS rating scale and the motor function outcome measures.

Results: Statistically significant correlations were observed between the STS scale and the motor function outcome measures. Patients with higher STS scores were stronger on MVIC ($r = 0.775$, $p = 0.001$), walk further in 6MW ($r = 0.803$, $p = 0.001$), faster in 25FWT ($r = -0.720$, $p = 0.001$), and TUG ($r = -0.759$, $p = 0.001$).

Discussion and conclusion: This STS grading scale is a valid measure of gross LE motor function in ALS patients, and scoring correlates with functional impairment in a linear fashion. The test is easily administered in the clinic and is clinically descriptive of a patient's level of impairment. STS scale could be used as an outcome measure for therapeutic efficacy and clinical decision-making.

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P16 A DESCRIPTION OF PHYSICIAN PAIN MANAGEMENT PRACTICE IN ALS

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

Background: Pain in ALS occurs in up to 70% of patients at some time during their course of the disease. The under-recognition of pain in ALS has been the subject of a recent editorial. Research on pain in ALS and guidelines for its treatment are needed.

Objectives: To describe North American physicians' practices of, and perspectives on, pain management in ALS.

Methods: A self-reported and descriptive survey of pain management was designed and e-mailed to physician members of the Northeast ALS Consortium (NEALS) with a link to the anonymous survey.

Results: A 33% response rate was obtained. All 37 respondents were Neurologists who routinely assessed for pain in ALS Clinics. Pain assessments were performed by the Neurologist (92%), Nurse (58%), PT/OT (54%), and Medical Assistant (15%). Most commonly, open-ended questions were utilized to assess for pain (88.5%). Less commonly used was numeric rating scales (15%), visual analog scales (8%), and standardized questionnaires (4%). Management of pain was most frequently under the domain of the Neurologist (96%) and less frequently by palliative care (27%), pain management clinics (27%), and primary care providers (20%). Close to half of the sample indicated that ALS pain was of sufficient severity to impair quality of life. Most common types of pain seen in ALS patients were musculoskeletal (100%), muscle cramps and spasms (96%), generalized/poorly defined (61%), and radicular (34%). Pharmacologic pain management included use of anti-spasticity medications (100%), agents for neuropathic pain (100%), non-narcotic analgesics (96%), narcotic analgesics (75%) and trigger point injections (21%). Non-pharmacologic pain management therapies included PT (100%), complementary/alternative management (50%), biofeedback (8%) and cognitive behavioural and mindfulness therapies (each 4%). Top perceived barriers to effective management of

pain in ALS were lack of effective medications (70%), limited information regarding best practices for pain management in ALS (57%) and physician training and experiences in pain management (40%).

Discussion and conclusion: Similar to perceptions of patients with ALS, their professional care providers found pain to be a significant component of the disease. As expected, musculoskeletal pain and muscle cramps were common, but the frequent perception by physicians that pain often is generalized and poorly defined points to a need to better understand patients' perception of such pain, and to a need for physicians to understand the overlap with suffering, depression, hopelessness, and other psychological pain. The perceived lack of effective medications may relate to a poor understanding of the aetiopathogenesis of this type of generalized pain. Many physicians are seeking best practices for pain management, and better training to accomplish this. A more complete knowledge of physical pain and psychological pain and suffering in patients with ALS should be considered as important steps toward addressing these needs.

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P17 SELF-REPORTED MEASURES PREDICT SURVIVAL IN ALS

THAKORE N, PIORO E

Cleveland Clinic, Cleveland, Ohio, USA

Email address for correspondence: thakorn@ccf.org

Keywords: survival, DeltaFS (ΔFS), ALSFRS-R

Objective: To explore the effect of self-reported measures on survival in ALS.

Methods: ALSFRS-R and other self-reported measures were prospectively collected on tablet devices in outpatient waiting rooms using software developed in-house (Knowledge Program). Cox proportional hazard models identified predictors of survival.

Results: Of 931 patients with ALS seen over a 7-year period, 256 died with a median survival of 726 days (about 2 years). Rate of decline of ALSFRS-R (ΔFS) was computed from 2 ALSFRS-R measures at least 45 days apart in 297 patients. Median ΔFS was 0.59 points/month. Higher ΔFS was strongly predictive of poorer survival, with a hazard ratio of 2.02 per point (CI 1.70–2.41). On univariate analyses, additional predictors were increasing age (HR 1.04 per year, CI 1.03–1.06); lower initial ALSFRS-R (HR 1.03 per point, CI 1.01–1.05); lower initial bulbar and respiratory ALSFRS-R subscores (HR 1.1 and 1.09 respectively per point); predominant bulbar/respiratory dysfunction (HR 1.79, CI 1.21–2.64); lower initial EQ-5D (HR 1.16 per 0.1 change, CI 1.10–1.22); higher initial PHQ9 (HR 1.07 per point, CI 1.05–1.10); and lower initial body weight and BMI (HR 1.03 per kg and 1.1 per unit respectively). ΔFS, age, PHQ9, predominant bulbar/respiratory dysfunction, and weight/BMI emerged as significant independent predictors of survival in stepwise regression. A predictive survival model is presented.

Discussion and conclusion: Self-reported measures have utility in predicting outcomes and for stratification of ALS patients into clinical trials.

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P18 BIOLOGICAL FOLLOW-UP IN AMYOTROPHIC LATERAL SCLEROSIS: CREATININE DECREASE AND FERRITIN INCREASE ARE PREDICTIVE OF A POOR PROGNOSIS

PATIN F[1,2], CORCIA P[1,3], MADJI HOUNOUM B[1], VEYRAT-DUREBEX C[1,2], RESPAUD E[1], PIVER E[4], BENZ DE BRETAGNE I[2], VOURC'H P[1,2], ANDRES CR[1,2], BLASCO H[1,2]

[1]*Unité mixte de recherche U930, Institut National de la Santé et de la Recherche Médicale, Université François-Rabelais, équipe "Neurogénétique et Neurométabolomique", Tours, France,* [2]*Laboratoire de Biochimie et de Biologie Moléculaire, Hôpital Bretonneau, Centre Hospitalier Régional Universitaire de Tours, Tours, France,* [3]*Service de Neurologie, Centre Hospitalier Régional Universitaire de Lille, Lille, France,* [4]*Laboratoire de Biochimie, Hôpital Trousseau, Centre Hospitalier Régional Universitaire de Tours, Tours, France*

Email address for correspondence: patin.franck@gmail.com

Keywords: biomarkers, creatinine, ferritin

Background: Clinical parameters are insufficient to predict disease duration in ALS patients, and biological follow-up is often limited to monitoring hepatic toxicity of Riluzole.

Objectives: To determine the progression of 13 routine biochemical markers in ALS and their correlation with clinical parameters at diagnosis and during disease evolution.

Methods: We retrospectively collected biological data from systematic routine monitoring of 216 patients: creatinine; ASAT; ALAT; GGT; ALP; iron; ferritin; transferrin; total cholesterol; HDL-cholesterol; LDL-cholesterol; and triglycerides, both at diagnosis and every 3 months until 30 months. We also noted the following clinical data: site at onset; age at onset; ALSFRS; FVC at diagnosis. We chose the annual decline of ALSFRS-R and survival as markers of disease progression. Firstly the relationship between clinical parameters and biological parameters at diagnosis was evaluated using Student t-test and spearman rho correlation. Secondly, the evolution of the biological parameters levels (early and late variation was measured at 6th and 24th months respectively) was evaluated and the relationship between the evolution over 24 months of these biological parameters and the disease progression using ANOVA test on repeated measures was assessed. Finally, the relationship between biological parameters at the diagnosis and their early variation (over 6 months) on the disease progression was evaluated. Correlation test of Spearman was used to analyze the annual decline of ALSFRS-R and survival analysis was performed using a Cox multivariate analysis.

Results: First, we observed that, at diagnostic time, creatinine was correlated with ALSFRS-R ($p < 0.0001$) and ferritin was linked to FVC ($p = 0.0168$). We highlighted a significant variation of creatinine ($p = 0.0166$) and ferritin ($p = 0.0306$) over the 24 first months of disease. Interestingly, we showed that early and long-term creatinine decrease (ANOVA: $p < 0.002$ and Cox model: $p = 0.004$) and ferritin increase (ANOVA: $p < 0.002$ and Cox model: $p = 0.0101$) were linked to disease progression. Elevated LDL/HDL ratio at diagnosis was found to be predictive of a shorter survival time (Cox model: $p = 0.0028$). Final multivariate model for survival analysis, including age at disease onset, early variation of ferritin, Forced Vital Capacity at diagnosis and diagnostic delay, showed that early variation of ferritin was an independent factor to predict patients' survival ($p = 0.004$[illegible]

ratio was correlated with diagnostic delay, and data were insufficient for early variation of creatinine, so LDL/HDL and early variation of creatinine were not included in multivariate analysis.

Discussion and conclusion: Ferritin and creatinine seem to vary over time, and this variation was linked to disease progression. This study provides new evidences suggesting that these routine biological biomarkers could be evaluated in patient's follow-up. To our knowledge, it is the first study describing the effect of time on these biomarkers and the relation between their evolution and disease progression

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P19 WHAT DO ALS PATIENTS DIE OF? – AN AUTOPSY STUDY OF 70 ALS PATIENTS

BURKHARDT C[1], NEURWIRTH C[1], SOMMACAL A[2], ANDERSEN P[3], WEBER M[1]

[1]*ALS Clinic/Neuromuscular department Kantonsspital St. Gallen, St. Gallen, Switzerland,* [2]*Department of Pathology Kantonsspital St. Gallen, St. Gallen, Switzerland,* [3]*Institute of Pharmacology and Clinical Neuroscience, Umeå University, Umeå, Sweden*

Email address for correspondence: christian.burkhardt@kssg.ch

Keywords: causes of death, autopsy, survival

Background: Death is the definitive hallmark of amyotrophic lateral sclerosis and primary endpoint in most treatment studies. Despite its importance limited data are available about the definitive causes of death in ALS nowadays. Previous autopsy studies (1) pointed out that defining the cause of death based solely on a clinical examination is not a reliable method to reveal the true cause of death. Treatment of our patients was according to the EFNS guidelines for patient care from 2005 (2). It is unclear if treatments such as non-invasive ventilation (NIV) or percutaneous gastrostomy (PEG) have an impact on the cause of death.

Objectives: The aim of this study was to gain a better understanding of causes of death in ALS patients and to investigate how these supportive treatments have an impact on the survival and the causes of death in ALS patients.

Methods: Seventy ALS patients were followed in our outpatient clinic and autopsied including a complete macroscopic and microscopic post mortem analysis between 2003 and 2014. Viscera for the pathological causes of death and relevant concomitant diseases were also studied. Neural tissue and CSF was stored for upcoming projects. Median time from point of death to autopsy was 4 h.

Results: In this study, the main cause of death was respiratory failure (69/70 patients). In 39/70, aspiration pneumonia and broncho-pneumonia led to death. 22//70 died of hypoxia and 5 patients requested assisted suicide inducing respiratory failure. Pulmonary embolism alone or in combination with pneumonia was detected in six. Both bulbar ($n = 3$) and spinal onset patients ($N = 3$) had embolism without any clear correlation to mobility status. A single patient died from a complication after PEG insertion. Average survival in patients using NIV was 7 month longer than without NIV and even more distinct in the NIV group comparing only limb onset patients. Bronchopneumonia was more frequent in patients using NIV versus non-NIV patients (19/38 versus 5/26, $p < 0.003$). The proportion of aspiration pneumonia was significantly lower in patients with PEG (7/43 versus 7/26, $p < 0.003$). PEG had no effect on survival or BMI at death. Genetic testing could be performed in 32 patients prior to death. Disease-causing mutations (*SOD1* or *C9orf72*) were found in about 1/4 of this cohort.

Discussion and conclusion: In this first autopsy study after establishing of the EFNS guidelines, NIV has a positive effect on survival but may be a risk factor for bronchopneumonia. PEG insertion lowers the risk of aspiration pneumonia but has no effect on survival. No correlation was observed between pulmonary embolism and ambulatory disability or site of onset.

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P20 MUSCLE ARCHITECTURE BY MEANS OF ECHOMYOGRAPHY, MULTIFREQUENCY ELECTRICAL IMPEDANCE MYOGRAPHY (MEIM) AND FORCE-TIME CURVE (F-TC) ANALYSIS IN ALS PATIENTS AS BIOMARKERS FOR PREDICTING MUSCLE DISEASE PROGRESSION

CORBIANCO S[1,3], BONGIOANNI P[1,2], TOSI C[3], D'ERAMO M[3], ROSSI B[1], DINI M[3,2]

[1]*Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy,* [2]*NeuroCare onlus, Pisa, Italy,* [3]*HuMoRe Lab, Pisa, Italy*

Email address for correspondence: bongioanni.paolo@gmail.com

Keywords: echomyography, MEIM, Force-time curve analysis

Background: The typical ALS disease course is characterized by progressive irreversible muscle wasting of limbs, torso, abdomen, and oropharynx, in the absence of muscle markers of disease progression. The aim of this study was to clarify if EchoIntesity (EI), EchoMuscle Thickness (EMT) and Multifrequency Electrical Impedence Myography (MEIM) are correlated with muscle force and clinical disease progression in ALS patients, and whether such analyses can be used as clinical biomarkers.

Methods: Nine male patients (mean age ± SD: 57.3 ± 9.5 yrs) with defined ALS according to the El Escorial criteria (1) were enrolled in our study. All patients were stable in pharmacological medication (50 mg riluzole twice a day); none received any steroid drug treatment. Force was measured both for biceps brachialis and tibialis anterior by a strain gauge system (Loumas Load Cell - Modena, Italy), and results were expressed in relation to a force peak (F) and course in 30 sec-1 (F-TC). MEIM was performed by an impedenzometer (DSMedical - Milan, Italy) that was calibrated each morning before measurements were made. In MEIM analysis (0 to 300 kHz) two source electrodes and two detecting electrodes were used to determine the Nyquist plot (Xc, Rz) for both biceps and tibialis muscles (2). Muscle ultrasonography (EI and EMT) was performed in real time by a 7.5-MHz linear array; gain, time-gain compensation and compression were kept constant (3). All patients were tested every 4 months and analyzed across a 12-month period.

Results: F and F-TC significantly ($p < 0.05$) decreased in all patients along the disease progression. EMT decreased and

EI increased significantly ($p < 0.05$) at the month12, but not at months 4 and 8. MEIM analysis, in particular at 50 kHz, pointed out significantly ($p < 0.05$) enhanced Rz and decreased Xc values month 8, and even more significantly ($p < 0.01$) both ones at month 12 ($p < 0.01$).

Discussion and conclusion: Data analysis suggests a significant correlation among MEIM, EI, EMT and disease progression, especially when the disease worsens rapidly. The Nyquist plot components are the earliest biomarkers for muscle decline during disease progression. Such findings might be related to the likely initial re-innervation as a factor for maintenance of almost normal values for EI and EMT parameters, whereas the MEIM values that directly come from the muscle cell derangement (intramuscular fibrous and fatty tissue) alone, across the disease progression, precociously highlight pathophysiological condition.

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P21 VIBRATION-INDUCED INHIBITION OF H-REFLEX IN ALS – A BIOMARKER FOR UPPER MOTOR NEURON DYSFUNCTION AND PREDICTOR OF FUNCTIONAL IMPAIRMENT

LEE MI[1], LANCE J[3], BURKE D[3], FITZPATRICK R[1], SIMON N[2], KIERNAN M[2]

[1]*Neuroscience Research Australia, Sydney, Australia,* [2]*Brain and Mind Research Institute, The University of Sydney, Sydney, Australia,* [3]*Institute of Clinical Neurosciences, Royal Prince Alfred Hospital and The University of Sydney, Sydney, Australia*

Email address for correspondence: m.lee@neura.edu.au

Keywords: H-reflex, vibration, upper motor neuron signs

Background: The diagnosis of ALS relies on clinical detection of concomitant upper motor neuron (UMN) and lower motor neuron (LMN) dysfunctions in the same limb or bulbar region. However, UMN signs in the limbs of ALS patients are difficult to assess clinically particularly if there is prominent neurogenic weakness.

Objectives: To investigate vibration-induced inhibition of the soleus H-reflex in patients with ALS and the results correlated with clinical UMN score (1) and Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS).

Methods: Soleus H-reflex studies were undertaken using a threshold-tracking paradigm in 35 ALS patients and 21 healthy controls. The tibial nerve at the popliteal fossa was electrically stimulated to elicit H-reflex in the soleus muscle. The electrical current needed to produce a H-reflex amplitude of approximately 10% of the maximal direct motor response (Mmax) was tracked using a semi-automated computer program (Q-Trac) and used as a surrogate marker of motoneuronal excitability and will be referred to as H-Th from here on. Soleus H-Th was compared between groups and across 3 conditions: during focal vibration (50 Hz) of the Achilles' tendon (Ach) or tibialis anterior (TA) and no vibration (control condition).

Results: Soleus H-reflex was absent in 7 ALS patients (20%, present in all controls). H-Th was higher in ALS patients (14 mA; $n = 28$) compared to controls (8.5 mA; $p = 0.0031$). In all controls, vibration of Ach and TA immediately abolished the H-reflex and the H-Th was elevated (by 30.7% and 24.7% respectively) to compensate for the vibration-induced inhibition. In contrast, ALS patients ($n = 23$) showed much smaller threshold changes during Ach and TA vibration (3.1% and 5.5% respectively; $p < 0.0004$). This was a consistent feature in all ALS patients irrespective of UMN signs (mean UMN score = 8, range between 0–16). ALSFRS was positively correlated with Soleus H-Th change during vibration of Ach ($r = 0.478$, $p = 0.002$) and TA ($r = 0.466$, $p = 0.022$), with the association stronger for bulbar onset ($r = 0.63$, $n = 11$, $p = 0.04$) and upper limb onset ALS patients ($r = 0.93$, $n = 7$, $p = 0.003$).

Discussion and conclusion: Vibration-induced inhibition of H-reflex is suppressed in ALS patients with and without clinically detectable UMN signs and may assist in identifying UMN pathology in patients referred with the possible diagnosis of ALS. Furthermore, the magnitude of H-reflex inhibition correlates with the level of functional impairment, particularly for bulbar and upper limb onset ALS.

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THEME 2 IMAGING, ELECTROPHYSIOLOGY AND MARKERS OF DISEASE PROGRESSION

P22 EVIDENCE FOR PERIPHERAL IMMUNE ACTIVATION IN AMYOTROPHIC LATERAL SCLEROSIS

CHEN X, GUO X, CHEN Y, SHANG H

West China Hospital, Sichuan University, Chengdu, Sichuan, China

Email address for correspondence: chenxueping0606@sina.com

Keywords: peripheral immune activation, disease progression

Background: There is evidence of the activity of immune system in the spinal cords of patients with amyotrophic lateral sclerosis (ALS), however; few studies to date have explored the status of peripheral immune response in ALS patients.

Methods: Blood from 284 ALS patients and 217 aged-match controls were evaluated, and parameters of T cell subset, humoral immunity, and complement system activation were observed.

Results: $CD4^+$T lymphocytes and circulating immune complexes (CICs) were significantly decreased, and component C3 was significantly increased in ALS patients compared with normal controls. Patients with severe or moderate impairment had a higher $CD4^+$T cell percentage and a lower IgG levels when compared to those with mild impairment. There was an inverse correlation between both $CD4^+$T cell percentage and revised ALS Functional Rating Scale (ALSFRS-R) score and disease duration, but the correlation was positive between both IgG level and ALSFRS-R score and disease duration among ALS patients. These correlations were gender-specific. This investigation demonstrated the existence of peripheral immune abnormalities in ALS patients.

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P23 URINARY P75 NEUROTROPHIN RECEPTOR EXTRACELLULAR DOMAIN AS A BIOMARKER OF SYMPTOMATIC DISEASE ONSET AND PROGRESSION

ROGERS M-L[1], WUU J[2], SHEPHEARD S[1], CHATAWAY T[1], SCHULTZ D[3], ANDERSON PM[4], BENATAR M[2]

[1]*Department of Human Physiology and Centre for Neuroscience, Flinders University, Adelaide, South Australia, Australia,* [2]*Neurology Department University of Miami, Miller School of Medicine, Miami, Florida, USA,* [3]*Neurology Department and MND Clinic, Flinders Medical Centre, Bedford Park, South Australia, Australia,* [4]*Institute of Pharmacology and Clinical Neuroscience, Umeå University, Umeå, Sweden*

Email address for correspondence: mary-louise.rogers@flinders.edu.au

Keywords: biomarkers, disease progression, pre-symptomatic

Background: There is an urgent need for robust biomarkers of ALS disease progression. We have previously shown that the urinary p75 neurotrophin receptor extracellular domain (p75NTRECD) concentration is higher in ALS patients compared to healthy controls (1).

Objectives: The goals of the study were to determine: (a) whether urinary p75NTRECD concentration is elevated in unaffected people at genetic risk for developing ALS; (b) when in the course of disease p75NTRECD levels begin to increase; (c) whether p75NTRECD levels are stable through the symptomatic phase of disease or whether levels change over time as disease progresses.

Methods: We studied 23 healthy control subjects (age 51 ± 13 years, 10 male), 29 ALS patients (age 63 ± 13 years, 14 male), and 24 pre-symptomatic *SOD1* mutation carriers (age 49 ± 11 years, 3 male) from the Pre-Symptomatic Familial ALS (Pre-fALS) study. Three pre-symptomatic individuals developed manifest disease during follow-up (phenoconverters); the other 21 have not phenoconverted for at least 6 months since last evaluation. Cross-sectional samples were assayed from all. Additional longitudinal samples (2–5 per person) were assayed from the 3 phenoconverters and 22 affected individuals; median times of collection of their first and last samples were, respectively, 9.9 (range 1.2–49.3) and 19.5 (range 5.8–66.4) months after symptom onset. Urinary p75NTRECD was quantified using a sandwich ELISA, with normalization against urinary creatinine concentration (1).

Results: As confirmed by previous findings, p75NTRECD levels were not associated with age or gender in healthy controls. In cross-sectional comparisons, mean ($\pm$ SD) p75NTRECD levels were comparable between controls (2.7 ± 0.8 pg/mg creatinine) and the pre-symptomatic group (3.0 ± 0.6) but were significantly higher among ALS patients (5.7 ± 2.5) compared to both other groups ($p < 0.001$). Longitudinal measurements of p75NTRECD in the 3 phenoconverters showed that p75NTRECD increased only *after* phenoconversion. Moreover, longitudinal measurements in the ALS affected group showed that, with rare exceptions, p75NTRECD increased as disease progressed ($p = 0.016$). p75NTRECD also correlated strongly with the ALSFRS-R ($p = 0.002$).

Discussion and conclusion: Cross-sectional data show that p75NTRECD is elevated, not in pre-symptomatic SOD1 mutation carriers, but only in affected individuals. Longitudinal data in three phenoconverters suggest that the increase occurs only after symptom onset. Combined, these findings indicate that urinary p75NTRECD is unlikely to be a useful biomarker of disease progression in the pre-symptomatic stage, but that it may still have diagnostic value in at-risk individuals who present with early and equivocal clinical manifestations. Most importantly, however, is that longitudinal data from affected individuals demonstrate the utility of p75NTRECD as a biomarker of disease progression in the symptomatic state.

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P24 CORTICAL ATROPHY IN THE ALS-FTD SPECTRUM

RATTI E[1,2], DOMOTO-REILLY K[2], HOCHBERG D[2], BRICKHOUSE M[2], CASO C[2], MURPHY A[1], CUDKOWICZ M[1], DICKERSON B[2]

[1]*Neurological Clinical Research Institute (NCRI), Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA,* [2]*Frontotemporal Disorders Unit, Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA*

Email address for correspondence: eratti@partners.org

Keywords: cortical thickness, magnetic resonance imaging (MRI), frontotemporal dementia (FTD)

Background: Amyotrophic lateral sclerosis-frontotemporal dementia (ALS-FTD) presents with the spectrum of heterogeneous clinical manifestations and pathologic findings of both ALS and FTD. In ALS, cortical atrophy is found primarily in the precentral gyrus with variable involvement of extra-motor cortices (fronto-temporal, cingulate, insula, parietal, occipital). FTD is characterized by predominant frontal lobe (dorsolateral, medial frontal, orbitofrontal, anterior cingulate) cortical atrophy in the behavioural variant and fronto-temporal-parietal atrophy in the language variant. There are limited evaluations of quantitative cortical thickness analysis in the full ALS-FTD spectrum within one study (1).

Objectives: To measure cortical atrophy in patients with ALS-FTD and compare with that of ALS and FTD, based on the hypothesis that ALS-FTD reflects cortical atrophy of the disease spectrum and, depending on clinical presentation, to a larger extent than isolated disease.

Methods: 3.0 Tesla MRI scans were acquired from 114 controls, 12 ALS-FTD patients, 10 ALS patients and 40 patients with FTD (including both language and behavioural variants). Quantitative cortical thickness analysis was performed using Freesurfer software.

Results: Compared with controls, ALS-FTD patients demonstrated atrophy in all portions of the prefrontal cortex, precentral gyrus, anterior cingulate, and anterior temporal cortex. Compared with controls, ALS patients present atrophy in the precentral gyrus, cingulate and parietal-occipital regions. Compared with controls, FTD patients demonstrated atrophy in all portions of the prefrontal cortex, anterior cingulate, anterior insula, and anterior temporal cortex. Comparisons among the three disease groups are ongoing.

Discussion and conclusion: In ALS-FTD, atrophy is prominent in the frontal cortical region in a pattern similar to that seen in FTD, and extends caudally to include the precentral gyrus. In ALS-FTD, atrophy in the anterior temporal cortical region is less prominent than in FTD. Compared to ALS, in ALS-FTD atrophy is less prominent in the precentral gyrus, but generally more diffuse and particularly increased in the pre-, medial- and orbito-frontal cortices and temporal region. Further work will investigate whether cortical thickness in a subset of these regions in patients with FTD or ALS alone could predict the development of ALS-FTD and inform about potential cortical and system vulnerability within the full ALS-FTD spectrum of disease.

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P25 PATTERNS OF CEREBRAL AND CEREBELLAR WHITE MATTER DEGENERATION IN ALS

BEDE P, ELAMIN M, BYRNE S, MCLAUGHLIN R, KENNA K, VAJDA A, FAGAN A, BRADLEY D, HARDIMAN O

Trinity College Dublin, Dublin, Ireland

Email address for correspondence: bedepeter@hotmail.com

Keywords: MRI, biomarkers, DTI

Background: The identification of a core pattern of white matter pathology in ALS is essential for the development of automated diagnostic protocols, such as those based on classifier analyses. However, the description of disease-defining white matter changes is confounded by the genetic, neuropsychological and clinical heterogeneity of ALS. Over forty diffusion tensor imaging (DTI) papers have been published in ALS to date, and while corticospinal tract and corpus callosum involvement are invariably highlighted, DTI studies are conflicting regarding the degree of extra-motor and cerebellar white matter degeneration.

Methods: We performed a comprehensive diffusion tensor imaging study with 42 healthy controls, 27 cognitively intact *C9orf72* negative ALS patients, and 9 ALS patients carrying the *C9orf72* hexanucleotide expansion. The patients tested negative for a comprehensive list of other known ALS causing gene mutations, such as *FUS, SOD1, TARDBP, ANG, VAPB, VCP, OPTN, SETX* and *ALS2*. Tract-based statistics were performed for fractional anisotropy, axial, mean and radial diffusivity at both whole-brain and cerebellar level. Results were corrected for multiple comparisons, age, gender, and disease duration.

Results: Fractional anisotropy, axial, radial and mean diffusivity analyses highlighted a pattern of bilateral corticospinal tract, mid-body corpus callosum, cerebellar and occipital white matter pathology. The three dimensional representation of white matter integrity changes represents a key spatial signature of ALS pathology. Contrary to previous studies, limited frontotemporal white matter changes were identified in the *C9orf72* negative ALS group. Based on the identified cerebellar white matter changes, a region-of-interest based DTI analysis was also carried out, confirming significant and extensive bilateral white matter degeneration in the cerebellum in the *C9orf72* negative, non-demented patient cohort. *C9orf72* positive patients demonstrated multiple cerebellar white matter regions with decreased axial, mean and radial diffusivity compared to *C9orf72* negative patients.

Conclusion: Cerebellar white matter degeneration is an important feature of ALS and is likely to contribute to the heterogeneous motor and neuropsychological deficits observed clinically.

Acknowledgements: This study was supported by the Elan Fellowship in Neurodegeneration, the Health Research Board (HRB-Ireland), the Research Motor Neuron (RMN-Ireland) foundation, the European Community's Seventh Framework Programme (FP7/2007–2013) under grant agreement no. [259867] (EUROMOTOR), and the EU-Joint Programme for Neurodegeneration (JPND) SOPHIA project.

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P26 SUBCORTICAL STRUCTURES IN AMYOTROPHIC LATERAL SCLEROSIS

WESTENENG H-J[1], VERSTRAETE E[1], WALHOUT R[1], SCHMID RT[1], HENDRIKSE J[2], VELDINK JH[1], VAN DEN HEUVEL M[3], VAN DEN BERG LH[1]

[1]*Department of Neurology,* [2]*Department of Radiology,* [3]*Department of Psychiatry, Brain Center Rudolf Magnus, University Medical Center Utrecht, Utrecht, The Netherlands*

Email address for correspondence: h.j.westeneng@umcutrecht.nl

Keywords: magnetic resonance imaging, basal ganglia, longitudinal

Background: Subcortical involvement in amyotrophic lateral sclerosis (ALS) is known from histopathologic studies, nuclear imaging and diffusion tensor imaging but detailed structural changes of subcortical structures (deep gray matter, hippocampal subfields, ventricles) were sporadically reported *in vivo* (1).

Objective: To assess the involvement of deep gray matter, hippocampal subfields and ventricular changes in patients with amyotrophic lateral sclerosis (ALS), both cross-sectionally and longitudinally.

Methods: 112 ALS patients and 60 healthy subjects participated. High-resolution T1-weighted images were acquired using a 3T MRI scanner. 39 ALS patients underwent a follow-up scan. Volumetric and shape analyses were performed to characterize the subcortical structures in healthy controls and ALS patients. Measures were correlated with clinical parameters and longitudinal changes were assessed.

Results: At baseline, reduced hippocampal volumes (left: $p = 0.007$; right: $p = 0.011$) and larger inferior lateral ventricles (left: $p = 0.013$; right: $p = 0.041$) were found in ALS patients compared to healthy controls. With regard to the hippocampal subfields, the presubiculum of the left hippocampus showed the largest difference between patients and controls (left: $p = 0.009$; right: $p = 0.052$). Longitudinal analyses demonstrated a significant decrease in volume of the right cornu ammonis 2/3 and 4/dentate gyrus and left presubiculum ($p = 0.002$, $p = 0.045$ and $p < 0.001$), and a significant increase in the ventricular volume in the lateral (left: $p < 0.001$; right: $p < 0.001$), 3rd ($p < 0.001$) and 4th ($p = 0.001$) ventricles. Smaller basal ganglia, limbic structures and larger ventricles at baseline were univariately associated with shorter survival ($p = 0.003$, $p = 0.029$ and $p = 0.007$), and larger ventricles were also associated with a lower ALSFRS-R at baseline ($p = 0.021$).

Discussion and conclusion: ALS patients show signs of neurodegeneration of subcortical structures and ventricular enlargement. Subcortical involvement is progressive and correlates with clinical parameters in ALS, highlighting its role in the neurodegenerative process in ALS.

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P27 THE SPECTRUM OF BASAL GANGLIA PATHOLOGY ACROSS THE ALS-ALS/FTD CONTINUUM

MACHTS J[1], LOEWE K[2,3], JAKUBICZKA S[4], KAUFMANN J[2], ABDULLA S[1,2], PETRI S[5], DENGLER R[5], HEINZE H-J[2,6], SCHOENFELD MA[2,6], VIELHABER S[1,2], BEDE P[7]

[1]*German Center for Neurodegenerative Diseases (DZNE), Magdeburg, Germany,* [2]*Department of Neurology,* [3]*Department of Knowledge and Language Processing,* [4]*Institute for Human Genetics, Otto-von-Guericke University, Magdeburg, Germany,* [5]*Department of Neurology, Hannover Medical School, Hannover, Germany,* [6]*Leibniz Institute for Neurobiology, Magdeburg, Germany,* [7]*Academic Unit of Neurology, Trinity College Dublin, Dublin, Ireland*

Email address for correspondence: judith.machts@dzne.de

Keywords: basal ganglia, cognition, frontotemporal dementia (FTD)

Background: Recent histopathology and neuroimaging studies have demonstrated significant subcortical grey matter involvement in ALS. From a clinical perspective, frontostriatal, nigrostriatal, and corticobasal circuitry dysfunction are likely to contribute to the unique cognitive, behavioural, extrapyramidal, and sensory deficits observed in ALS. However, very little is currently known about the level of basal ganglia involvement along the ALS-ALS/FTD continuum and the neuropsychological manifestations of basal ganglia changes in ALS.

Objectives: To characterize the spectrum of basal involvement across the ALS-ALS/FTD continuum, using multifaceted quantitative imaging techniques.

Methods: 67 ALS patients and 39 age-, gender- and education-matched controls (HC) gave informed consent to undergo detailed neuropsychological testing, comprehensive genotyping, and high-resolution 3 Tesla MR imaging. Participants were categorised based on diagnosis and cognitive performance into four groups; HC ($n = 39$), ALS patients without cognitive and/or behavioural impairment (ALS-NCI; $n = 42$), ALS patients with cognitive and/or behavioural impairment (ALS-Plus; $n = 18$), and ALS patients with comorbid frontotemporal dementia (ALS-FTD; $n = 7$). Given the strong imaging signature of the C9orf72 repeat expansions, only non-mutation carriers were selected for this study. Volumetric, vertex, average signal intensity and voxel-wise density values were calculated for seven subcortical structures (thalamus, amygdala, nucleus accumbens, hippocampus, caudate nucleus, pallidum, putamen) using automated segmentation tools. Voxel-wise, vertex-wise and group-wise comparisons were carried out between the study groups correcting for multiple comparisons (TFCE, $p < 0.05$), age, and total intracranial volume.

Results: Vertex-wise shape analyses revealed focal changes in the amygdala between ALS-NCI patients and controls. ALS-

Plus patients had focal volume loss in the left amygdala and right caudate nucleus in comparison to ALS-NCI patients. In comparison to healthy controls ALS-Plus patients also showed significant volume reductions and vertex changes in bilateral hippocampi. Considerable volumetric, shape, and density changes were identified in all subcortical structures for the ALS-FTD group as compared to controls, ALS-NCI, and ALS-plus groups. However, it should be noted that the differences between the ALS-FTD and ALS-plus groups were not as pronounced as those seen between the ALS-FTD and ALS-NCI groups. Overall, a spectrum of incremental basal ganglia pathology was present across the four study groups. This trend has been unequivocally demonstrated by all of the complementary imaging modalities of the study, including shape, volume and density analyses.

Conclusion: Our findings support the notion that the extent of subcortical grey matter pathology in C9orf72-negative ALS is closely associated with cognitive and behavioural changes, and represent a continuum along which the conditions of ALS-NCI, ALS-plus and ALS-FTD lie. Our results also indicate that, in addition to cortical grey and subcortical white matter measures, imaging studies of cognition in ALS need to take basal ganglia degeneration into account.

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P28 MICROSTRUCTURAL CHANGES ACROSS DIFFERENT STAGES OF DISEASE IN AMYOTROPHIC LATERAL SCLEROSIS

TROJSI F[1], PICCIRILLO G[1], CAIAZZO G[1], FEMIANO C[1], CORBO D[1], CRISTILLOV[1], FERRANTINOT[1], DAMIANO R[1], ESPOSITO F[2], MONSURRÒ MR[1], TEDESCHI G[1]

[1]*Second University of Naples, Naples, Italy, Italy,* [2]*University of Salerno, Baronissi, Salerno, Italy, Italy*

Email address for correspondence: dr.gpiccirillo@gmail.com

Keywords: diffusion tensor imaging, voxel-based morphometry

Objectives: Neurodegenerative process in amyotrophic lateral sclerosis (ALS) has been proven to involve several cortical and subcortical brain regions within and beyond motor areas. However, how ALS pathology spreads progressively across different stages of disease is still unknown. In this cross-sectional study we aimed to identify white (WM) and gray matter (GM) patterns of degeneration in a large population of ALS patients in different stages of disease.

Methods: We investigated 54 ALS patients, divided into 3 subsets according to the clinical stage, and 18 age and sex-matched healthy controls, using tract-based spatial statistics (TBSS), diffusion tensor imaging (DTI) and voxel-based morphometry (VBM) analyses.

Results: ALS cases in stage 2A (at diagnosis) were characterized by GM and WM impairment of left motor and premotor cortices and brainstem at ponto-mesenchephalic junction. ALS patients in clinical stage 2B (with impairment of two functional regions) exhibited decreased fractional anisotropy (FA) ($p < 0.001$, uncorrected) and increased mean (MD) and radial diffusivity (RD) ($p < 0.001$, uncorrected) in the left cerebellar hemisphere and brainstem precerebellar nuclei, as well as in motor areas, while GM atrophy ($p < 0.001$, uncorrected) was detected only in the left inferior frontal gyrus and right cuneus. Finally, ALS patients in stage 3 (with impairment of three functional regions) exhibited decreased FA and increased MD and RD ($p < 0.05$, corrected) within WM underneath bilateral pre and postcentral gyri, corpus callosum midbody, long associative tracts and midbrain, while no significant clusters of GM atrophy were observed.

Discussion and conclusion: Our findings reinforce the hypothesis that the neurodegenerative process propagates along the axonal pathways and develops beyond motor areas from early stages, involving progressively several frontotemporal regions and their afferents and efferents, while the detection of GM atrophy in earlier stages and its disappearance later may be the result of reactive gliosis.

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P29 FUNCTIONAL CONNECTIVITY HALLMARKS IN ALS

LOEWE K[1,2], MACHTS J[3], STOPPEL C[1,4], KAUFMANN J[1], ABDULLA S[1,3], KOLLEWE K[5], PETRI S[5], DENGLER R[5], HEINZE H-J[1,6], HOPF J-M[1,6], BORGELT C[7], VIELHABER S[1,3], SCHOENFELD MA[1,6]

[1]*Department of Neurology,* [2]*Department of Knowledge and Language Processing, Otto-von-Guericke University, Magdeburg, Germany,* [3]*German Center for Neurodegenerative Diseases, Magdeburg, Germany,* [4]*Department of Psychiatry and Psychotherapy, Charité-Universitätsmedizin, Berlin, Germany,* [5]*Department of Neurology, Hannover Medical School, Hannover, Germany,* [6]*Leibniz Insitute for Neurobiology, Magdeburg, Germany,* [7]*European Centre for Soft Computing, Mieres (Asturias), Spain*

Email address for correspondence: judith.machts@dzne.de

Keywords: functional connectivity, resting-state fMRI, graph analysis

Background: Recent studies place ALS and frontotemporal dementia (FTD) on opposite poles of one clinical continuum. Although ALS-related changes in extra-motor areas have been observed using structural MRI, such studies have found only limited involvement of temporal lobe regions. Reports on functional connectivity changes in ALS were also rather inconclusive with respect to the continuum hypothesis. Here, we investigated resting-state functional connectivity using a novel approach based on whole-brain voxel-level graphs to map ALS-related brain network changes at the level of individual voxel pairs.

Methods: We investigated patients with classical ALS ($n = 64$) and age-, gender- and education-matched controls ($n = 38$). All subjects underwent detailed neuropsychological assessment. Resting-state fMRI scans were acquired at 3 Tesla ($TR = 2200$ms, isotropic 3.5 mm voxels). Subject-specific connectivity graphs were constructed by defining gray matter voxels as nodes and establishing weighted edges by estimating internodal functional connectivity in terms of Pearson correlation between the nodes' associated time-series. Edge-level t statistics were computed across graphs to assess functional connectivity differences between study groups using directional two-tailed t tests. The resulting graph of statistics Gt was thresholded in order to identify voxel pairs exhibiting significant changes ($FDR \leq 0.175$).

Results: Neuropsychological deficits in ALS were minimal and limited to the executive and verbal memory domains. Prominent clusters of decreased functional connectivity in ALS were found in motor-related areas (bilateral pre- and postcentral gyrus) characterized by many affected long-range connections. Along with the motor system another functional system, namely the temporo-occipital cortex, exhibited extensive changes in connectivity. Most strikingly, extensive patterns

of decreased temporo-occipital connectivity spread from the medial and inferior temporal lobes up to the middle occipital lobes. In contrast to the affected motor connections, the temporal lobe involvement was mostly confined to intra-temporal and temporo-occipital connections (both intra- and interhemispheric). While far less pronounced, the analysis also revealed sparse patterns of increased connectivity in association with ALS incorporating mostly short-range connections within frontal, parietal, occipital, and temporal regions.

Conclusion: The observed decreased motor functional connectivity is consistent with previously reported structural damage of motor related areas in ALS. Since the patients only exhibited slight neuropsychological deficits, the extent of connectivity changes in the temporal lobes was, however, unexpected. Patterns of increased functional connectivity detected in prefrontal and parietal areas could reflect increased regulatory processes that may serve to compensate for the fronto-executive deficits frequently encountered in patients with ALS. These findings match the patterns of cerebral degeneration typically observed in FTD and support the idea that ALS and FTD are part of one clinical continuum. The presented voxel-level connectivity analysis appears to be very sensitive to pathological changes in neurodegenerative diseases such as ALS.

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P30 *IN VIVO* TRANSFER OF PATHOLOGY SPREADING PATTERNS BY DTI ANALYSIS IN ALS

KASSUBEK J, MÜLLER H-P, DEL TREDICI K, BRETTSCHNEIDER J, PINKHARDT E, LULÉ D, BÖHM S, BRAAK H, LUDOLPH AC

Dept. of Neurology, University of Ulm, Ulm, Germany

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

Keywords: diffusion tensor imaging, fractional anisotropy, fiber tracking

Introduction: Diffusion tensor imaging (DTI) is a well-established tool to identify ALS-associated patterns of brain alterations at the group level. Recently, a neuropathological study in ALS has shown that ALS may disseminate in a regional sequence in four disease-related patterns (1). The present study shows the application of a new methodological DTI-based approach to automatically analyze *in vivo* fiber structures that are prone to be involved at each neuropathological pattern of ALS (2).

Methods: Two data samples, consisting of 130 (n = 130) DTI data sets acquired at 1.5T from patients with ALS (n = 78) and from controls (n = 52) as well as 55 DTI data sets at 3.0T from patients with ALS (n = 33) and from controls (n = 22), were analyzed by a tract of interest (TOI)-based fiber tracking approach to analyze five tracts that become involved during the course of ALS: the corticospinal tract (stage 1), the corticorubral and the corticopontine tracts (stage 2), the corticostriatal pathway (stage 3), the proximal portion of the perforant path (stage 4), and two reference pathways.

Results: The statistical analyses of TOIs by tractwise fractional anisotropy statistics (TFAS – (3)) showed differences between ALS patients and controls for all tracts investigated. As the disease stage, with corresponding fiber tract involvement increased, the significance level of the comparisons at group level was lower. Data analysis at the individual level allowed for a categorization into ALS patterns. Both the clinical phenotype as assessed by ALS-FRS-R and the disease duration correlated significantly with the resulting staging scheme.

Discussion and conclusion: In summary, the TOI-based technique allowed for individual analysis of predefined tract structures. That way, *in vivo* imaging of the disease patterns in ALS has become feasible. This approach might enlarge the spectrum of potential non-invasive surrogate markers as a neuroimaging-based read-out for clinical trials in ALS.

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P31 NEURAL CORRELATES OF SUSTAINED ATTENTION IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS: AN EVENT RELATED POTENTIALS STUDY

OLIVARES A[1], IGLESIAS-FUSTER J[2], CUSPINEDA-BRAVO E[1], ZALDÍVAR T[1], LARA-FERNANDEZ G[1], SOTO-LAVASTIDAA[1], GUTIÉRREZ-GIL J[1], SÁNCHEZ-LÓPEZ J[1], MACHADO-CURBELO C[1]

[1]*Neurology and Neurosurgery Institute, Havana, Cuba,* [2]*Cuban Center for Neuroscience, Havana, Cuba*

Email address for correspondence: ana.olivares@infomed.sld.cu

Keywords: event related potentials (ERPs), cognitive impairment, sustained attention

Background: Several electrophysiological studies have provided evidence for extra-motor areas, demonstrating subclinical cognitive deficits in patients with Amyotrophic Lateral Sclerosis (ALS) (1–2). Auditory Event Related Potentials (aERPs) could characterize the cerebral information processing across the evolution of this neurological disease. Besides temporal dynamics, the estimation of the brain sources of such activity becomes critical. The Brain Electrical Tomography (BET) has been revealed as a suitable methodology for estimating those sources. The inclusion of the Bayesian Model Averaging (BMA) have improved the estimation's accuracy and reduced the appearance of spurious generators, creating an adequate framework for locating and characterizing the cognitive function in ALS patients.

Objectives: (1) To determine and localize sources of electrical cognitive activities using aERPs (N100, MMN, and P3b); (2) To identify differences in topography, latency and amplitude in components between groups.

Methods: BAEPs were recorded for hearing assessment. P3 component was also recorded on 15 patients with ALS by using a paradigm previously validated in 30 healthy volunteers using 32 EEG derivations. The amplitude and latency for the component obtained were measured. P3 Grand Average was computed and electrical sources were estimated by means of Bayesian Model Averaging (BMA) approach in both groups (patients and healthy controls). Clinical variables, depression and cognitive status were also evaluated with standardized scales for both groups.

Results: Normal BAEPs were present in all groups. A P3b component was observed in all ALS patients. No significant differences for latencies between patients and controls were found. Significantly, lower amplitude in P3 component ($p < 0.05$) was found in ALS group compared with healthy controls. Significantly lower activation was also found in the estimated source generators of both hemisphe

superior frontal and left middle frontal gyri, supra-temporal cortex) in ALS patients as compared with healthy controls.

Discussion and conclusion: The automatic, pre-attentive processing of auditory stimuli seems to be preserved in ALS. Decreased P3 amplitude indicates the presence of reduced sub-clinical attention deficits in ALS patients. This suggests a pathological involvement beyond the motor areas. The attention impairments found here evince the dysfunction of the frontal network and could be reflecting an early stage of fronto-temporal dementia. Finally, the combination of ERPs and source analysis represent a convenient technique to detect and assess sub-clinical impairment in patients with severe loss of motor function.

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P31.5 COMBINING DIFFUSION TENSOR IMAGING AND PERIPHERAL NERVE ULTRASOUND TO DISCRIMINATE BETWEEN ALS SUBTYPES

ABDULLA S[1,2], LÖWE K[4], SCHREIBER S[1], KAUFMANN J[1], DEBSKA-VIELHABER G[1], MACHTS J[3], DANNHARDT-STIEGER V[1], PETRI S[2], KOLLEWE K[2], SCHREIBER F[5], HEINZE H-J[1,4], DENGLER R[2], SCHOENFELD AM[1,4], NESTOR PJ[3], VIELHABER S[1,3]

[1]*Otto-von-Guericke University Magdeburg, Magdeburg, Germany,* [2]*Hannover Medical School, Hannover, Germany,* [3]*German Center for Neurodegenerative Diseases, Magdeburg, Germany,* [4]*Leibniz Institute for Neurobiology, Magdeburg, Germany,* [5]*Institute of Control Engineering, Braunschweig, Germany*

Email address for correspondence: abdulla.susanne@mh-hannover.de

Keywords: Diffusion tensor imaging, high-resolution ultrasound, classifier construction

Background: Diffusion tensor imaging (DTI) has successfully been used to investigate ALS-related changes in the central nervous system (CNS). Many studies reported, for example, decreased white matter integrity in the corticospinal tract (CST). Consequently, DTI has been proposed as a potential biomarker to support clinical diagnosis. While recent results look promising in principle, the reliability of DTI-based diagnosis seems currently insufficient for practical use. To enhance discriminative performance, one would ideally integrate information from complementary diagnostic modalities during classifier construction.

Objective: In a recent study, we observed ALS-related thinning of different peripheral nerves based on cross-sectional area (CSA) measurements using high-resolution ultrasound (1). Here, we combined DTI-based measures of the CST with CSA of the upper limb peripheral nerves to improve imaging-based discrimination between different ALS subtypes and healthy controls.

Methods: 51 patients with ALS (14 classic, 10 upper motor neuron dominant (UMND), 14 lower motor neuron dominant (LMND), 8 bulbar, and 5 primary lateral sclerosis (PLS)) and 18 healthy controls underwent diffusion-weighted MRI at 3T and peripheral nerve ultrasound in order to determine the fractional anisotropy (FA) in the white matter underneath the motor cortex, posterior limb of the internal capsule, cerebral peduncle, pons and medulla oblongata as well as the CSA of the N. medianus and the N. ulnaris. Statistical comparisons were conducted using analysis of variance (ANOVA). Classifier construction was carried using linear discriminants after dimensionality reduction based on principal component analysis. Separate classifiers were constructed using FA, CSA, and both FA and CSA data, respectively.

Results: Compared to controls, ALS patients exhibited significant, distally pronounced reductions of the ulnar nerve CSA across all subtypes with the exception of PLS. In addition, DTI data revealed significant differences between ALS subtypes with pronounced impairment of the CST in UMND and PLS and modest impairment in LMND patients. Classification performance was considerably higher exploiting both central and peripheral data compared to using either modality alone. Most notably, UMND and PLS patients could be accurately separated.

Discussion and conclusion: Combining central and peripheral data acquired through DTI and ultrasound, respectively, leads to improved discrimination not only between ALS and healthy controls, but also between ALS subtypes. This speaks to the complementary role of both methods regarding their contribution to classification performance. The potential of combining both imaging methods is particularly evident in the reliable separation of UMND and PLS patients, since early differentiation of these subtypes remain a difficult problem in clinical practice.

P32 LONGITUDINAL NEUROIMAGING MODELS IN ALS AND OTHER NEURODEGENERATIVE CONDITIONS

SCHUSTER C, HARDIMAN O, BEDE P

Academic Unit of Neurology, Trinity College Dublin, Dublin, Ireland

Email address for correspondence: schustec@tcd.ie

Keywords: presymptomatic, multiple time point, non-linear progression

Background: ALS is characterised by relentless functional decline. Accordingly, patterns of clinical and pathological spread; longitudinal cognitive and motor changes have been extensively studied. Nevertheless, the majority of imaging studies in ALS are cross-sectional, and relatively few longitudinal studies have been published to date.

Objectives: To comprehensively review the methodology, statistical models, follow-up intervals of high-impact longitudinal studies in neurodegeneration and assess their applicability to ALS. The overall aim of this review was to develop a sensitive ALS-specific longitudinal neuroimaging model that can be utilised to study the natural disease trajectory of ALS.

Methods: Longitudinal imaging studies were searched from 1990 to 2014 using PubMed. The main search terms "longitudinal" and "MRI" were utilised in combination with one of the following Keywords: amyotrophic lateral sclerosis; motor neuron disease; Alzheimer's disease; Huntington disease; Parkinson's disease; frontotemporal dementia. Additional searches for presymptomatic studies and healthy ageing were also conducted. Only articles published in English were reviewed. Longitudinal studies of multiple sclerosis and clinical trials were excluded. Studies were individually reviewed f

attrition rates, follow-up intervals, statistical models, reference groups and their ability to capture longitudinal changes.

Results: Based on the above search criteria, a total of 280 longitudinal imaging papers were identified: 21 in ALS; 156 in Alzheimer's disease; 21 in Huntington's disease; 26 in Parkinson's disease; 13 in frontotemporal dementia; and 43 publications in healthy ageing. The identified longitudinal imaging papers included diffusion, structural, spectroscopy and functional imaging studies, whole brain as well as region-of-interest studies. A wide range of simple and complex statistical models were utilized to highlight longitudinal changes. Three main approaches were identified; direct comparison of first and second time-points, analysis of (annualized) percentage change and mixed effect linear models. While follow-up interval was the shortest in ALS studies (3–6 months), attrition rates were higher in ALS studies compared to other neurodegenerative conditions. Healthy controls were the most frequently used reference group with only few studies using neurological controls.

Discussion and conclusion: There is a clear need for well-designed longitudinal studies in ALS, not only to understand the natural disease trajectory of the disease, but also to reliably measure potential drug-effects in pharmaceutical studies. The notion of using the patient's own previous data sets as reference data makes individual patient inferences possible. Given the significant changes captured at diagnosis, presymptomatic longitudinal imaging studies are likely to contribute to our understanding of premorbid ALS pathophysiology. Our results suggest that mixed effect linear statistical models are best suited to highlight progressive pathological changes.

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P33 CORTICAL DYSFUNCTION DISTINGUISHES PRIMARY LATERAL SCLEROSIS FROM HEREDITARY SPASTIC PARAPARESIS

GEEVASINGA N[1], MENON P[1], SUE CM[1], KUMAR KR[2], NG K[1], KIERNAN MC[1], VUCIC S[1]

[1]*University of Sydney, Sydney, Australia,* [2]*Royal North Shore Hospital, Sydney, Australia*

Email address for correspondence: nimesh84@hotmail.com

Keywords: upper motor neuron disorders, spastic gait disorders, transcranial magnetic stimulation

Background: In clinical practice when patients present with a spastic gait, difficulty ambulating and weakness in the lower limbs, it may be difficult from a clinical examination alone to differentiate between primary lateral sclerosis (PLS), hereditary spastic paraparesis (HSP) and amyotrophic lateral sclerosis (ALS). Distinguishing between PLS and HSP is clearly important for patient management, with implications for genetic counselling and disease prognosis.

Objective: The aim of the present study was to develop a novel diagnostic algorithm by combining threshold tracking transcranial magnetic stimulation (TMS) techniques together with clinical characteristics in order to facilitate the differentiation of PLS from HSP in a clinical setting.

Methods: Studies were undertaken on a cohort of 14 PLS and 13 HSP patients with genetically confirmed mutations in the spastin (SPG4) gene. Results were compared to 82 amyotrophic lateral sclerosis (ALS) patients and 37 aged matched controls.

Results: Bulbar dysfunction was significantly more frequent in PLS patients (PLS 64%; HSP 0%), while urinary urgency (PLS 29%; HSP 69%) and sensory dysfunction (PLS 0%; HSP 92%) were more frequent in HSP. The age of disease onset was significantly greater in PLS ($p < 0.0001$). Motor cortex inexcitability was more frequent in PLS (PLS 71%, ALS 24%; HSP 0%), and the resting motor threshold was increased in PLS ($F = 6.499$, $p < 0.0001$). Short interval intracortical inhibition (SICI) and cortical silent period (CSP) duration were significantly reduced in PLS when compared to HSP (SICI, $p < 0.01$; CSP $p < 0.001$) and controls (SICI, $p < 0.01$; CSP $p < 0.001$), but were comparable to ALS. Combining clinical and TMS abnormalities into a novel PLS diagnostic index reliably differentiated PLS from HSP ($p < 0.001$).

Discussion and conclusion: The combination of clinical features and TMS abnormalities reliably distinguished PLS and HSP in a clinical setting, potentially leading to more accurate diagnosis and better patient management.

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P34 ASSOCIATION BETWEEN RECTUS ABDOMINIS DENERVATION AND VENTILATION DYSFUNCTION IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

ZHANG H, ZHANG S, XU Y, FAN D

Peking University Third Hospital, Beijing, China

Email address for correspondence: dsfan2010@aliyun.com

Keywords: ventilation, rectus abdominis, needle EMG

Objective: Neurogenic changes of EMG of rectus abdominis (RA) muscles were regarded as an evidence of lesion in lower motor neuron involved in thoracic cord in amyotrophic lateral sclerosis (ALS). Denervation of RA was examined to detect association with ventilation dysfunction in ALS.

Methods: Clinical data including ALSFRS and Forced vital capacity (FVC) of 128 patients with sporadic ALS in Department of Neurology of PKU Third Hospital from 2009 to 2013 was collected. Standard needle EMG was recorded from at least three limbs, sternocleidomastoid and RA. Only spontaneous activity (fibrillation potentials (fib) and positive sharp waves (psw)) and pattern of recruitment were studied in RA. The differences of EMG changes of RA between patients with and without FVC > 80% were analyzed. Of the total, there were 33 patients with an ALSFRS score less than 4 in the respiratory subset. The relationship of fib-psw of RA and dyspnea was also observed.

Results: The mean FVC in the 128 ALS patients was 83.4 + 17.1% (range 45% –131%) of predicted. FVC > 80% accounted for 79 patients (mean ALSFRS score 33.9 + 4.1) and FVC < 80% for 49 (mean ALSFRS score 30.1 + 6.2). Compared with patients with normal FVC (60/79, 75.9%), fib-psw in RA showed significant difference for FVC < 80% (47/49, 95.9%). Moreover, there were more spontaneous potentials of RA in patients with symptom of dyspnea (32 /33, 97.0%) than those without (75 /95, 78.9%).

Conclusion: Spontaneous potentials of RA were associated with ventilation dysfunction as well as dyspnea in ALS patients. The hypothesis was supported that diaphragm and RA were involved concomitantly in ALS. Respiratory function tests, such as FVC, should be performed when fib-psw of RA were detected.

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P35 AN ELECTROPHYSIOLOGICAL EVALUATION OF PALMOMENTAL REFLEX IN ALS AND FTD PATIENTS

ALVISI E[1,2], FONTANA D[1], VERSIGLIA V[1], MARCHETTA L[1], MONTINI A[1], FRESIA M[1], CERONI M[3,2], LOZZA A[1], MOGLIA A[1,2], SANDRINI G[2,4], ALFONSI E[1]

[1]*Electrophysiology Department,* [2]*University of Pavia* [3]*General Neurology Department,* [4]*Neurorehabilitation Department- National Institute of Neurology C. Mondino Foundation, Pavia-ITALY*

Email address for correspondence: elena.alvisi.doc@gmail.com

Keywords: electrophysiology, biomarkers, palmomental reflex (PMR)

Background: Palmomental reflex (PMR) is a primitive reflex normally seen in early childhood; rarely it may persists later in life unless in presence of diseases affecting frontal lobes. Because of the continuum between amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), frontal impairment identification in ALS is an important target. In order to achieve a better diagnostic sensitivity we used an electrophysiological method to better assess the presence or the absence of this reflex.

Objectives: To assess 1) the different sensitivity between a classical clinical and an electrophysiological PMR detection in a cohort of FTD and ALS patients; 2) the possible different stimulation intensity able to detect its presence; 3) the ipsi and/or controlateral reflex presentation; 4) the difference in latency to onset

Methods: The PMR presence was assessed with an EMG study (2 recording channels) using subcutaneous needle electrodes applied to the mentalis muscle bilaterally after the delivery of single rectangular electric pulses with 0.2 msec duration, with an intensity between 6 and 30 mA applied in the palmar cutaneous area localized above abductor pollicis brevis. 30 ALS patients with and without cognitive impairments and 10 FTD patients according to El Escorial revised, Awaji, Strong and Neary criteria were included in the study. The main involvement of the first or of the second motorneuron at onset in both groups, and the presence or absence of cognitive impairment in ALS patients was analysed.

Results: PMR was clinically identified in 32% in ALS and in 33.3% in FTD patients, with an electrophysiological approach in about 80% of both disorders. ALS patients with a prominent first motorneuron involvement have a PMR evocation at a lower intensity stimulus (14, 25+/− 7, 44 vs 21,77 +/−7,08 mA; $p = 0.02$) which ALS-FTD compared with FTD patients demonstrate a more frequent reflex presence ipsi and controlaterally. The correlation between the short latency to onset, and the impairment of executive functions was statistically significant.

Discussion and conclusion: This electrophysiological tool can predict the early onset of PMR and suggests a prominent first motorneuron involvement in the disease progression. In addition this study supports the hypothesis of possible cognitive involvement in ALS patients in early stages of disease.

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P36 MODULATION OF FASCICULATION POTENTIAL FREQUENCY IN ALS

DE CARVALHO M[1], TURKMAN A[2], PINTO S[1], SWASH M[1,3]

[1]*Instituto de Medicina Molecular and Institute of Physiology, Faculty of Medicine,* [2]*Centro de Estatística e Aplicaçoes, Faculty of Sciences, University of Lisbon, Lisbon, Portugal,* [3]*Departments of Neurology and Neuroscience, Royal London Hospital, Queen Mary University of London, London, UK*

Email address for correspondence: mamedemg@netcabo.pt

Keywords: benign fasciculation syndrome, fasciculations, spinal muscle atrophy

Background: Fasciculation is an important clinical feature in ALS. The role of fasciculation potentials (FPs) in the electrodiagnostic process has been recently upgraded (Awaji criteria). In spite of large numbers of papers regarding their origin, morphology and stability, their modulation by external interventions has not been studied. However, clinicians often tap muscles when ALS is suspected, believing that this might excite fasciculations.

Objective: To test the effect of voluntary muscle contraction, lower motor neuron activation by motor nerve stimulation, and sensory nerve stimulation as possible modulators of FP frequency.

Methods: We studied the first dorsal interosseus muscle (FDI) of 31 ALS patients in whom this muscle had normal strength (ALS5), 23 ALS patients with slightly weak FDI (MRC = 4, ALS4), 10 patients with benign fasciculation syndrome (BFS) and 6 patients with spinal muscle atrophy (SMA). FP frequency was recorded thorough surface electrodes in 2 minute recordings. Following a baseline recording, FP frequency was evaluated in the subsequent randomized conditions: baseline; immediately after a 1 minute maximal isometric contraction; immediately after application of a 1Hz train of 50 supramaximal stimuli to the distal ulnar nerve; immediately after stimulation of the sensory branch of the radial nerve (at twice cutaneous threshold, 20 Hz, 600 stimuli). The baseline frequency was normalized and the percentage change evaluated by Wilcoxon Signed Rank Test (corrected $p < 0.05$ was considered as significant).

Results: Adapted neurophysiological index (amplitude × F-wave frequency) was significantly lower in ALS4 than ALS5. Baseline recording was reliable as no difference in FP frequency was found between runs ($p = 0.64$). FPs frequency at baseline was similar in the different conditions. There was a significant increase in FP frequency immediately after sensory radial nerve electrical stimulation in ALS4 ($p = 0.01$), but other modulatory interventions had no effect. In SMA an increase in FP frequency was observed following sensory stimulation, but this did not reach statistical significance. In BFS no intervention changed FPs frequency.

Conclusion: In ALS, peripheral electrical stimulation within the corresponding dermatome (radial nerve) increased the

probability of FP discharge in FDI when the muscle was weak. Activation of Ia afferent fibers thus probably increased motor lower neuron excitability. Our results suggest that surviving motor neurons show increased excitability and increased likelihood of a response to input from sensory nerves representing homologous spinal segments. These data support the concept that motor neuron soma excitability influences FP discharge in weak muscles with predominant distally evoked FPs. Our results are consistent with clinical practice and suggest a method for measurement of lower motor neuron excitability in ALS.

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P37 PHASE NUMBERS AND DURATION OF FASCICULATION POTENTIALS (FPS) HAVE NEGATIVE CORRELATION WITH MUSCLE STRENGTH IN ALS PATIENTS – A QUANTITATIVE ANALYSIS

BOKUDA K[1,2], SHIMIZU T[1,2], KIMURA H[1], YAMAZAKI T[1], SEKIGUCHI T[1], KAWATA A[1], WATABE K[2], TANAKA K[2], NAKANO I[1]

[1]*Department of Neurology, Tokyo Metropolitan Neurological Hospital, Fuchu, Tokyo, Japan,* [2]*Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan*

Email address for correspondence: kotabkd@gmail.com

Keywords: electromyogram, fasciculation potential, quantitative analysis

Background: In recent years, the importance of FPs in electrophysiological diagnosis of ALS is emphasized as shown in Awaji criteria 2008. In particular, pattern alterations of FPs such as their instability and complexity are said to be characteristic of ALS, and attract attention in terms of their relation to disease progression. However, there are only few quantitative analyses on the form of FPs seen in ALS, especially in advanced stage.

Objectives: The purpose of this study was to investigate the quantitative relation of FP-form changes with disease progression of ALS, especially those patients showing in muscle weakness.

Methods: A total of 36 consecutive patients with sporadic ALS who underwent needle electromyography (nEMG) in our hospital from Oct. 1, 2013 to March 31, 2014 were recruited (19 men and 17 women, mean age of 66 years at nEMG, range 34–87 years). In each patient, all FPs from four muscles (trapezius, biceps brachii, vastus medialis, tibialis anterior) were quantitatively analyzed on duration, amplitude, number of phases and frequency, and the relations with muscle strength were assessed. In this study, muscle strength was defined as the three groups: normal (MRC 5); slightly weak (MRC 4); severely weak (MRC 0–3).

Results: In total, 596 FPs were analyzed. Duration of FPs and the number of FP phases were negatively correlated ($p < 0.05$) with muscle strength in three out of the four muscles (trapezius, biceps brachii, tibialis anterior) and in only one muscle (biceps brachii) respectively. The amplitude was not correlated with muscle strength. The number of FP phase showed a positive correlation ($p < 0.05$) with FP duration in three muscles (trapezius, biceps brachii, tibialis anterior) and with amplitude in three muscles (trapezius, biceps brachii, vastus medialis). But we found no correlation between the amplitude and duration of FPs.

Discussion and conclusion: It has been said that reinnervation to denervated muscles brings about increase in phase number, amplitude and duration of FPs is considered to reflect reinnervation of muscles particularly in an early or middle stage of muscle weakness in ALS. This study has shown that phase number and the duration of the FPs increase as the muscles weakness progresses in ALS patients, even in advanced stage. It indicates that reinnervation is still occurring in severely weak muscle, in ALS patients.

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P38 THE KINECT-BASED REACHABLE WORKSPACE FOR MEASURING UPPER EXTREMITY FUNCTION IN ALS

OSKARSSON B[1], KURILLO G[2], JOYCE N[1], NICORICI A[1], DE BIE E[1], MCDONALD C[1], BAJCSY R[2], HAN J[1]

[1]*University of California, Davis, Sacramento, California, USA,* [2]*University of California at Berkeley, Berkley, California, USA*

Email address for correspondence: bjorn.oskarsson@ucdmc.ucdavis.edu

Keywords: Kinect, reachable workspace, outcome measure

Background: Previously we have shown that the low-cost Kinect based system can be accurately and reliably used to measure RSA as compared to a complex motion capture system (1). Using this Kinect based methodology we have shown marked differences between normal volunteers and patients with other neuromuscular diseases including facioscapulohumeral muscular dystrophy, Duchenne muscular dystrophy, and Pompe disease (2).

In this study, we present pilot data on the use of a vision-based sensor system and customized software, developed to capture three-dimensional (3D) upper extremity motion in ALS. We believe that this Kinect-based 3D reachable workspace area (RSA) could be an effective outcome measure.

Objective: To evaluate whether the Kinect-measured RSA as an outcome measure is sensitive enough to detect a difference between an ALS cohort vs controls.

Methods: Using the Kinect single stereo-camera system we recorded subjects arm movements in 4 horizontal and 4 vertical directions to cover their whole RSA, in less than 1 minute. We compared the arms of 10 ALS subjects to 40 healthy controls.

Results: All ALS subjects were able to perform the testing. The range of total RSA was 0.04–0.80, which represents severe impairment and normal function, respectively. The Kinect-based measure differed significantly between the ALS sample and the controls ($p < 0.01$ using Welsh's two sample T-test).

Discussion and conclusion: The Kinect-measured RSA is diminished in many ALS patients. As an outcome measure it holds promise as a quick, affordable, painless, and clinically meaningful continuous variable. The measure could be of particular use in trials specifically targeting the upper extremity/cervical spinal cord. We plan to conduct a longitudinal study with a larger sample to further examine its sensitivity, validity, and reliability.

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P39 DIAGNOSTIC USEFULNESS OF SPLIT-HAND INDEX USING MOTOR UNIT NUMBER INDEX (MUNIX) IN AMYOTROPHIC LATERAL SCLEROSIS

SONY-R[2], KIM D-G[2], SHIN J-Y[1], KIM S-M[1], HONG Y-H[3], PARK K-S[2], SUNG J-J[1], LEE K-W[1]

[1]*Seoul National University Hospital, Seoul, Republic of Korea,* [2]*Seoul National University Bundang Hospital, Seong-nam si, Republic of Korea,* [3]*Boramae Hospital, Seoul, Republic of Korea*

Email address for correspondence: chungmoon@hanmail.net

Keywords: MUNIX, split hand phenomenon, split index

Background: In amyotrophic lateral sclerosis (ALS), there is dissociated atrophy of the hand muscles. The abductor pollicis brevis (APB) and first dorsal interosseus (FDI) muscles are more affected than abductor digiti minimi (ADM). Some attempts have been made to use split hand phenomenon in diagnosis of ALS using compound muscle action potentials (CMAP).

Motor unit number index (MUNIX) is a reproducible electrophysiologic technique used to reflect the number of motor units in ALS patients. In this study we investigate whether split-hand index using MUNIX (SI_{MUNIX}) could be more sensitive than split-hand index using CMAPs (SI_{CMAP}) in ALS patients.

Methods: 21 clinically definite and probable ALS patients participated in this study. NCS and MUNIX study were performed for bilateral APB, FDI, and ADM. Ten age matched were enrolled. SI_{MUNIX} and SI_{CMAP} were compared between ALS and control. SI_{MUNIX} was calculated as $(MUNIX_{FDI}{*}MUNIX_{APB})/MUNIX_{ADM}$. SI_{CMAP} was calculated as $(CMAP_{FDI}{*}CMAP_{APB})/CMAP_{ADM}$. 42 limbs from ALS patients were classified by CMAP amplitude as normal CMAP and low CMAP groups, for further sub group analysis.

Results: There was 8 definite and 13 probable ALS according to El Escorial Criteria. Age was not significantly different with controls. (59.6 ± 11.5, 56.9 ± 10.2) Disease duration of normal CMAP ALS limbs was 6 ± 3.2 months; while in the low CMAP ALS limbs duration was 11.4 ± 9.3 months ($p < 0.05$).

SI_{MUNIX} and SI_{CMAP}: CMAP and MUNIX of each muscle were significantly lower in ALS group compared with controls ($p < 0.01$). SI_{MUNIX} and SI_{CMAP} also significantly lower in ALS group ($p < 0.001$, $p < 0.001$). We divided arms of ALS patients in a two group; Normal CMAP and low CMAP group. Both low CMAP ALS group and normal CMAP ALS group showed significantly low SI_{MUNIX} compared with control, respectively ($p < 0.001$, $P < 0.05$). SI_{CMAP} was also lowered in low CMAP ALS group than control ($p < 0.001$). However, There was no difference between normal CMAP ALS group and control in SI_{CMAP}.

Conclusion: SI_{MUNIX} might be useful in detection of split-hand phenomenon in early stage of ALS patients without CMAP abnormality than SI_{CMAP}.

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P40 AMYOTROPHIC LATERAL SCLEROSIS AFFECTS CORTICAL AND SUBCORTICAL ACTIVITY UNDERLYING MOVEMENT EXECUTION AND INHIBITION

KOLLEWE K[1], KRÄMER U[2], COLE D[3], HELDMANN M[2], SAMII A[4], DENGLER R[1], PETRI S[1], MÜNTE T[2], MOHAMMADI B[2,4]

[1]*Medical School Hannover, Hannover, Germany,* [2]*University of Lübeck, Lübeck, Germany,* [3]*University of Zurich, Zurich, Switzerland,* [4]*International Neuroscience Institute (INI), Hannover, Germany*

Email address for correspondence: kollewe.katja@mh-hannover.de

Keywords: fMRI, inhibition, motorcontrol

Introduction: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by loss of upper and lower motor neurons. Evidence suggests that ALS additionally affects other brain areas including premotor cortex and supplementary motor area. We studied movement execution and inhibition in ALS patients using a stop-signal paradigm and functional magnetic resonance imaging (fMRI).

Methods: Seventeen ALS patients and 17 age-matched healthy controls were included. Participants performed a stop-signal task that required responding with button press to a right- or left-pointing black arrow (go-stimuli). In stop-trials, a red arrow (stop-stimulus) was presented shortly after the black arrow indicating to withhold the prepared movement. A total of 512 trials were presented (25% stop-trials). Magnetic-resonance images were acquired on a 3-T Siemens Magnetom Scanner.

Results: Patients had marginally higher reaction times in go-trials, but did not differ significantly in their inhibition performance. ALS patients showed however stronger inhibition-related activity in inferior, superior and middle frontal gyrus as well as in putamen and pallidum. Error-related activity on the other hand was found to be stronger in healthy controls, particularly in the insula bilaterally. In the go-task we found execution related increase of the activated area in the contralateral sensorimotor cortex in ALS patients.

Discussion and conclusion: ALS patients and controls showed specific differences in neural networks underlying motor execution, motor inhibition and error monitoring. The results provide further evidence for altered prefrontal functions in ALS.

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P41 REGIONAL PATTERNS OF 'RESTING-STATE' FUNCTIONAL HYPERCONNECTIVITY IN ALS: THE ASSOCIATION WITH SEQUENTIAL SPREADING

RÖLL I, GORGES M, LULÉ D, BRETTSCHNEIDER J, DEL TREDICI K, BRAAK H, LUDOLPH AC, KASSUBEK J

Dept. of Neurology, University of Ulm, University of Ulm, Germany

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

Keywords: intrinsic functional connectivity, resting state, diffusion tensor imaging

Background: Autopsy-controlled studies in ALS proposed a sequential spreading of the underlying TDP-43 pathology (1). Recently the *in vivo* transfer of the neu

patterns by use of diffusion-tensor-imaging (DTI) has been performed (2). Based upon the DTI findings, we have further developed the imaging approach in this study by the complementary investigation of intrinsic functional connectivity functional MRI (ifc-fMRI) in order to trace the spreading of pathology in defined intrinsic functional connectivity networks (ICNs).

Methods: The 3.0 T rs-fMRI protocol for 36 ALS patients and 34 healthy controls comprised 200 volumes, 3.0×3.0 mm^2 in-plane resolution, 4.0 mm slice thickness; TR and TE were 2000 ms and 30 ms, respectively. The post processing followed standardized procedures including motion correction, MNI normalization, spatial filtering (7mm FWHM), temporal linear detrending and bandpass filtering ($0.01 < f < 0.08$ Hz). ICNs were identified utilizing the seed-based approach (3) using Tensor Imaging and Fiber Tracking (TIFT) software. The investigated ICNs comprised the motor (neuropathological pattern 1); frontoparietal and frontal executive (pattern 2); basal ganglia (pattern 3); and hippocampal network (pattern 4); in addition the visual ICN was used as a reference network.

Results: ALS patients compared with healthy controls demonstrated a large-scale distributed pattern of increased functional connectivity (hyperconnectivity) in all networks, with the exception of the reference network. More specifically, the hyperconnectivity pattern was observed in large parts of the respective ICN and spatially extended into adjacent brain structures preferentially towards more frontal portions. Moreover, the disease patterns as observed *in vivo* by DTI significantly correlated with increasing functional connectivity in the ICNs.

Conclusion: Ifc-fMRI provides a promising approach to functionally trace pathological spreading in ALS. The observed functional hyperconnectivity pattern might point towards compensatory mechanisms to reduced structural integrity or might reflect a spreading loss of inhibitory cortical influence, as previously discussed (4). The pattern of ALS-associated functional connectome alterations seems to follow the pattern observed in neuropathological studies.

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P42 SEXUAL DIMORPHISM IN ALS

BEDE P, ELAMIN M, BYRNE S, MCLAUGHLIN R, KENNA K, VAJDA A, FAGAN A, BRADLEY D, HARDIMAN O

Trinity College Dublin, Dublin, Ireland

Email address for correspondence: bedepeter@hotmail.com

Keywords: MRI, biomarkers, DTI

Background: Gender differences have been repeatedly described in ALS regarding age of onset, site of onset, prognosis and cognitive profile. Sexual dimorphism in healthy populations is also well established. Nevertheless, imaging studies of ALS continue to allocate male and female participants into admixed study groups, and frequently omit to correct for gender in their statistical models which may be an important confounding factor.

Objectives: To explore neuroanatomical differences between female and male ALS patients in the context of sexual dimorphism in healthy controls.

Methods: Fourteen female ALS patients, 13 male ALS patients, 22 healthy male controls and 20 healthy female controls were recruited into a comprehensive neuroimaging study. Cortical thickness measurements and diffusion tensor imaging (DTI) were utilized to explore gender specific anatomical vulnerability. A general liner model was used to explore if age-adjusted vertex wise cortical thickness, accounting for diagnosis, differ between female and male participants. A similar model was also used for DTI data, exploring gender effect on tract-based diffusivity measures, accounting for age and diagnosis.

Results: DTI analysis across all study groups revealed increased fractional anisotropy in association with male gender in the brain stem, cerebellum, fornix, thalamus, anterior forceps and corticospinal tracts accounting for diagnosis and age. While females showed a trend of higher age-adjusted cortical thickness in the right parietooccipital and left midfrontal regions, males demonstrated higher cortical thickness in the left lingual and left superior temporal regions, accounting for age and diagnosis. Correcting for age, significant multifocal white matter differences have also been identified between healthy male and female controls.

Conclusion: Sexual dimorphism is an overlooked and potentially confounding factor in admixed ALS neuroimaging studies. Given the significant pre- and post-morbid gender differences, we propose that ALS imaging studies should be strictly controlled for gender or, alternatively single gender studies should be considered.

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P43 RESTING STATE FMRI CONTINUUM BETWEEN AMYOTROPHIC LATERAL SCLEROSIS AND BEHAVIORAL VARIANT FRONTOTEMPORAL DEMENTIA

PICCIRILLO G[1], TROJSI F[1], DE STEFANO M[1], BUONANNO D[1], MONTELLA P[1], MONSURRÒ MR[1], ESPOSITO F[2], TEDESCHI G[1]

[1]*Second University of Naples, Naples, Italy, Italy,* [2]*University of Salerno, Baronissi, Salerno, Italy, Italy*

Email address for correspondence: dr.gpiccirillo@gmail.com

Keywords: resting state fMRI, frontotemporal dementia (FTD)

Background: Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are multisystem neurodegenerative disorders, which have been found highly related, occupying two poles of a disease spectrum, with a predominance of motor dysfunction at one end and cognitive symptoms at the other. Although neuroimaging techniques have been proven potentially useful to unravel divergent and overlapping features between these syndromes, structural and functional magnetic resonance imaging (MRI) correlates of the hypothetical continuum between ALS and behavioural variant Frontotemporal Dementia (bvFTD) have been poorly explored

Methods: Using resting state functional magnetic resonance imaging (RS-fMRI), we investigated resting state functional MRI (RS-fMRI) connectivity changes in a series consecutive patients with ALS and bvFTD compared with healthy controls (HCs), looking for connectivity pattern divergence or overlap between the two disorders.

Results: Compared to controls, we found decreased RS-fMRI signals within sensorimotor (SMN), right fronto-parietal (R-FPN), and salience (SLN) networks in both patient groups. Specifically, with regard to SMN, while in ALS patients the suppression was strongly confined to the precentral gyri, in bvFTD patients the effect started from the precentral gyri and extended towards the temporal cortex. Within the default mode network (DMN), divergent connectivity patterns were observed, with RS-fMRI signals in the posterior cingulate cortex enhanced in bvFTD patients and suppressed in ALS patients.

Discussion and conclusion: Our findings show the potential impact of RS-fMRI as a noninvasive technique to explore whole-brain functional connectivity in degenerative diseases like ALS and FTD, whose neurobiological mechanisms are only partially known and, for some aspects, probably converging. Moreover, our results suggest that ALS and bvFTD share common connectivity patterns, corroborating the theory that they probably constitute different phenotypical expressions of the same neurodegenerative process.

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P44 FRONTOTEMPORAL CONNECTIONS AND ATTENTIONAL CONTROL IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS: A 3T DIFFUSION TENSOR IMAGING AND NEUROPSYCHOLOGICAL STUDY

CHRISTIDI F[1], ZALONIS I[1], KYRIAZI S[2], RENTZOS M[1], KARAVASILIS E[2], FERENTINOS P[3], ZOUVELOU V[1], WILDE EA[4], EVDOKIMIDIS I[1]

[1]*Department of Neurology, Eginition Hospital, Medical School, National & Kapodistrian University, Athens, Greece,* [2]*Radiology Research Unit, Medical Imaging Department, Evgenidion Hospital,* [3]*Department of Psychiatry, Eginition Hospital, Medical School, National & Kapodistrian University, Athens, Greece,* [4]*Departments of Physical Medicine and Rehabilitation, Neurology and Radiology, Baylor College of Medicine and the Michael E. DeBakey Veterans Affairs Medical Center, Houston, Texas, USA*

Email address for correspondence: christidi.f.a@gmail.com

Keywords: diffusion tensor imaging, attentional control

Background: Advanced neuroimaging and neuropsychological studies have revealed enough sensitivity to characterize *in vivo* the extra-motor involvement in patients with amyotrophic lateral sclerosis (ALS) (1). Pathological extra-motor changes have been found in the uncinate fasciculus (UF) (2–3), a major frontotemporal white matter tract that contributes to memory, word retrieval, emotional processing, problem solving and attentional control; these cognitive processes are often impaired in ALS (4).

Objectives: The present study investigates the relations between UF integrity and attentional control in patients with ALS, using DTI at 3T and standardized neuropsychological measures.

Methods: 21 patients with definite or probable ALS (11 with spinal onset, 6 with bulbar onset, and 4 with both spinal and bulbar onset) according to the El Escorial Criteria (5) and 11 healthy demographically-related participants were included in this study. Fractional anisotropy (FA), apparent diffusion coefficient (ADC), axial and radial diffusivity (D_A and D_R, respectively) were the DTI metrics examined. Attentional control was evaluated with Trail Making Test-part B (TMT-B) and Stroop Neuropsychological Screening Test (SNST). The level of statistical significant was set at a = 5%.

Results: Patients with ALS showed significant bilateral reduction of D_A in the UF as compared to controls (left UF, $p = 0.046$; right UF, $p = 0.021$). Within patients with ALS, TMT-B significantly correlated with left (FA, $r = -0.541$, $p = 0.011$; ADC, $r = 0.657$, $p = 0.001$; D_A, $r = 0.624$, $p = 0.002$; D_R, $r = 0.630$, $p = 0.002$) and right (FA, $r = -0.496$, $p = 0.022$) UF. Also, SNST significantly correlated with left (FA, $r = -0.541$, $p = 0.011$; ADC, $r = -0.620$, $p = 0.003$; D_A, $r = -0.497$, $p = 0.022$; D_R, $r = -0.615$, $p = 0.003$) and right (D_R, $r = -0.434$, $p = 0.050$) UF. Patients' corticospinal tract integrity did not correlate with any UF or cognitive measures.

Discussion and conclusion: Patients with ALS showed microstructural alterations in UF, in accordance to the reported prefrontal and temporal white matter abnormalities. Disruption of the extra-motor tract of the UF may explain patients' attentional control impairment and is independent of corticospinal tract integrity. Advanced neuroimaging can detect *in vivo* UF changes in patients with ALS, with these microstructural changes being associated with attentional control.

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P45 PSYCHOTIC SYMPTOMS ARE RELATED TO CEREBELLAR-THALAMIC-CORTICO NETWORKS IN THE MOTOR NEURONE DISEASE-FRONTOTEMPORAL DEMENTIA CONTINUUM

DEVENNEY E[1,2], IRISH M[1,3], AHMED RM[1,2], MIOSHI E[1], HODGES JR[1,3], KIERNAN MC[2,4], HORNBERGER M[1]

[1]*Neuroscience Research Australia, Sydney, Australia,* [2]*Brain and Mind Research Institute, Sydney, Australia,* [3]*University of New South Wales, Sydney, Australia,* [4]*University of Sydney, Sydney, Australia*

Email address for correspondence: e.devenney@neura.edu.au

Keywords: MND-FTD continuum, psychosis, neu

Background: Psychotic features, in particular delusions and hallucinations, are prevalent in a subset of patients with MND0 and FTD, particularly those with TDP-43 pathology. Similarly, the *C9orf72* genetic mutation causes TDP-43 protein accumulation and many MND and FTD carriers present with psychosis. More recently imaging studies of the *C9orf72* mutation have demonstrated thalamic and cerebellar atrophy in addition to frontal and temporal atrophy. Together these findings suggest that the cerebellar-thalamic-cortico network may be involved in the generation of psychotic symptoms across the MND-FTD continuum.

Objective: To determine the neural correlates of psychosis in the MND-FTD continuum.

Methods: All patients (n = 42) and a group of matched healthy controls (n = 20) underwent T1 weighted MRI scans at presentation. In addition, each participant was subject to a detailed clinical interview, neuropsychological testing and functional disability assessment. Initially a composite score was generated to express the degree of psychosis present for each subject. This score was calculated from responses during clinical interview and the carer based delusions and hallucinations subcategories of the Neuropsychiatric Inventory (NPI). In a second phase we employed voxel based morphometry (VBM) techniques to identify areas of whole brain grey matter atrophy and subsequently to co-vary the degree of psychosis to regions of atrophy.

Results: Compared to controls the patient group exhibited significant atrophy across a variety of regions including bilateral frontal and temporal lobes, the cerebellum, basal ganglia and thalamus ($p < 0.001$ corrected). A significant association was found between thalamic, basal ganglia and cerebellar atrophy and psychotic symptoms across the MND-FTD continuum ($p < 0.001$ uncorrected).

Conclusion: This novel study suggests that atrophy involving the thalamus and its subcortical networks are critical for the generation of psychotic features in the MND-FTD continuum. This highlights the prevalence of delusions and hallucinations in this cohort and further advances our understanding of the complex brain networks involved. With future developments in pharmacology on the horizon, specific treatments aimed at disease burden in these regions may benefit this patient group.

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P46 PREFRONTAL INVOLVEMENT RELATED TO COGNITIVE IMPAIRMENT IN PROGRESSIVE MUSCULAR ATROPHY

RAAPHORST J[1,9], VANTOL M-J[4], GROOT P[5], ALTENA E[7,10], VAN DER WERF Y[7,8], MAJOIE C[5], VAN DER KOOI A[1], VAN DEN BERG LH[6], SCHMAND B[1,3], DE VISSER M[1], VELTMAN D[2]

[1]*Department of Neurology, University of Amsterdam, Amsterdam, The Netherlands,* [2]*Department of Psychiatry, VU University Medical Centre, Amsterdam, The Netherlands,* [3]*Department of Psychology, University of Amsterdam, Amsterdam, The Netherlands,* [4]*Neuroimaging Center, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands,* [5]*Department of Radiology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands,* [6]*Brain Center Rudolf Magnus Institute of Neuroscience, Department of Neurology, University Medical Center Utrecht, Utrecht, The Netherlands,* [7]*Netherlands Institute for Neuroscience, an Institute of the Royal Netherlands Academy of Arts and Sciences, Amsterdam, The Netherlands,* [8]*Department of Anatomy and Neurosciences, VU University Medical Centre, Amsterdam, The Netherlands,* [9]*Department of Neurology, Radboud University Medical Center, Nijmegen, The Netherlands,* [10]*Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK*

Email address for correspondence: joost.raaphorst@radboudumc.nl

Keywords: progressive muscular atrophy (PMA), verbal fluency, fMRI

Background: In patients with progressive muscular atrophy (PMA) non-motor cerebral involvement is disputed. Recently we have shown reduced white matter integrity of the prefrontal cortex in PMA, in part similar to amyotrophic lateral sclerosis (ALS). We hypothesized that PMA patients show non-motor cerebral involvement in frontal and temporal brain regions previously associated with fluency performance in ALS.

Objective: To examine brain activation patterns during verbal fluency performance in patients with PMA and ALS.

Methods: Functional MRI (fMRI) was used to examine the blood oxygenation level dependent (BOLD) response during letter and category fluency performance in 18 PMA patients, 21 ALS patients and 17 healthy control subjects, matched for age and education. fMRI results are reported at $p < 0.05$, Family Wise Error (FWE) corrected for multiple comparisons. We analyzed effects of performance, age related white matter changes (ARWMC) and regional brain volumes; all participants underwent neuropsychological investigation.

Results: Disease duration of patients with PMA (mean 26.0 months, SD 13.6) and ALS (22.2; SD 11.4) were comparable. PMA and ALS patients had mild to moderate disease severity and showed impaired letter fluency compared to controls. Between group analysis showed a main effect of group in the left inferior frontal gyrus (IFG, Brodmann area 45) during letter fluency which was unaffected by performance, ARWMC and IFG volume: PMA patients showed lower activation than controls but higher than that of ALS patients (ALS< PMA< HC; $p_{FWE} = 0.035$, Z-score 4.11; size = 11 voxels). A more caudal region in the IFG showed lower activation in PMA patients than controls during letter fluency performance (post-hoc test; $p_{FWE} = 0.026$). No activation differences were observed during the category fluency task.

Discussion and conclusion: Prefrontal activation abnormalities are related to an important clinical measure of executive dysfunction, in motor neuron disease patients with and without upper motor neuron signs. The presence of non-motor cerebral involvement related to cognitive dysfunction in PMA further supports the view that a proportion of PMA patients should be regarded as ALS.

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THEME 3 COGNITIVE AND PSYCHOLOGICAL ASSESSMENT AND SUPPORT

P47 COGNITIVE AND BEHAVIOURAL SYMPTOMS ALONG THE ALS SPECTRUM: DETECTION, DIFFERENTIATION AND PROGRESSION

HSIEH S[1], CAGA J[1], SHIBATA M[1], LESLIE F[2,5], DAVESON N[2], FOXE D[2], RAMSEY E[1], LILLO P[3], AHMED RM[2,1], DEVENNEY E[2,1], BURRELL J[2,5], HODGES JR[2,5], MIOSHI E[4,2], KIERNAN MC[1]

[1]*Brain and Mind Research Institute, Sydney, NSW, Australia,* [2]*Neuroscience Research Australia, Sydney, NSW, Australia,* [3]*Departamento de Neurologia Sur, Universidad de Chile, Santiago, Chile,* [4]*Department of Psychiatry, Cambridge University, Cambridge, UK,* [5]*University of New South Wales, Sydney, NSW, Australia*

Email address for correspondence: sharpley.hsieh@sydney.edu.au

Keywords: cognitive screening, frontotemporal dementia (FTD), behavioural symptomatology

Background: A brief clinical screening tool which can detect cognitive and behavioural symptoms in ALS is pertinent, particularly for patients with an adjunct frontotemporal dementia diagnosis (ALS FTD). The differentiation of ALS FTD from patients with subtle but definite cognitive or behavioural symptoms (ALS plus) and motor symptoms only (ALS pure) is also relevant in light of recent diagnostic criteria. Finally, the emergence of cognitive and behavioural symptoms with disease progression in ALS has important implications for disease management.

Objectives: To investigate the effectiveness of two validated screening tools in detecting, differentiating and elucidating the progression of cognitive and behavioural symptoms along the ALS spectrum: The Mini-Addenbrooke's Cognitive Examination (M-ACE), a brief cognitive screening tool, and the Motor Neuron Disease Behavioural Scale (MiND-B), a behavioural questionnaire.

Methods: ALS patients (n = 54) and controls (n = 45) were recruited from the Motor Neuron Disease Multidisciplinary Clinic at the Prince of Wales Hospital (MiND) and the Frontier Frontotemporal Dementia Research Group, Sydney, Australia. Comprehensive examination enabled classification of the participants into those with an adjunct diagnosis of FTD (ALS FTD; n = 25) and those without (n = 29) were further subdivided into ALS pure (n = 17) and ALS plus (n = 12) according to the Strong criteria (1).

Results: The M-ACE and MiND-B combined correctly classified 88% of ALS FTD patients. Moreover, almost all ALS FTD (> 90%) patients scored below the cut-off of 25/30 on the M-ACE. Neither one of the two screening tools alone differentiated between the three ALS cohorts: the MiND-B differentiated between ALS pure and ALS plus only whereas the M-ACE differentiated between the ALS FTD and those without the adjunct FTD diagnosis. Finally, Rasch modelling of M-ACE and MiND-B items revealed that cognitive and behavioural symptoms emerged synchronously with fluency, memory and apathy being more prominent early in ALS.

Discussion and conclusion: The M-ACE was useful for the detection of ALS FTD patients but did not differentiate between ALS pure and ALS plus groups. This was perhaps not surprising as ALS plus is currently defined according to behavioural symptomatology and executive dysfunction, both of which are not measured by the M-ACE. Interestingly, the severity of behavioural symptomatology did not differ between ALS plus and ALS FTD groups. This likely reflects the heterogeneity of the ALS FTD sample whereby some individuals had prominent behavioural symptoms whereas language impairment predominates in others.

The M-ACE and MiND-B combined detects ALS FTD patients, differentiates along the ALS continuum and offers insight into the progression of non-motor symptomatology in ALS.

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P48 THE COGNITIVE PROFILES OF ALS AND FTD SHOW CONSIDERABLE OVERLAP

BEELDMAN E[1], KLEIN TWENNAAR M[1], RAAPHORST J[1,2], DE HAAN R[3], DE VISSER M[1], SCHMAND B[1,4]

[1]*Department of Neurology, University of Amsterdam, Amsterdam, The Netherlands,* [2]*Department of Neurology, Radboud University Medical Centre, Nijmegen,* [3]*Clinical Research Unit, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands,* [4]*Department of Psychology, University of Amsterdam, Amsterdam, The Netherlands*

Email address for correspondence: e.beeldman@amc.uva.nl

Keywords: cognitive profile, frontotemporal dementia (FTD), meta-analysis

Background: Cognitive impairment is present in 30% of ALS patients and consists of deficits in executive functions, language, social cognition and memory. Based on the overlap of ALS and frontotemporal dementia (FTD), we aimed to test the hypothesis that the cognitive profiles are similar.

Objectives: To compare the results of a meta-analysis of the cognitive profile of FTD with an update of our previous meta-analysis of the cognitive profile of ALS.

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Methods: Embase, PsycInfo and Medline were searched for neuropsychological studies of behavioural variant FTD patients (bv-FTD) and age and education matched healthy volunteers. At least one validated neuropsychological test had to be used and means and standard deviations had to be presented. All tests were categorized in cognitive domains and effect sizes (Hedges' g) were calculated. The comparison of the cognitive profiles of FTD and ALS was performed using two different approaches. First, the overlap of the confidence intervals of the effect sizes of FTD and ALS was investigated. Second, the effect sizes and the confidence intervals were standardized and expressed as standard deviations from the mean effect size of FTD and ALS ((effect size single domain - mean effect size FTD or ALS) / SD of mean effect size FTD or ALS).

Results: We screened 7384 abstracts and evaluated 449 full text papers. 93 articles were included in the FTD meta-analysis (1855 FTD patients and 2005 controls). All cognitive domains (n = 12) showed significant effect sizes. The largest effect sizes in FTD were seen for social cognition, MMSE and fluency (1.61, 1.51 and 1.44, respectively). There was a considerable difference in effect sizes between FTD and ALS patients, ie, the largest effect size of ALS patients was 0.63, and there was no overlap of the confidence intervals of any of the cognitive domains. The comparison of the standardized effect sizes showed a considerable overlap for social cognition, fluency, executive functions, delayed and immediate verbal memory, visuoperception and the MMSE.

Discussion and conclusion: The cognitive profiles of bv-FTD (deficits in social cognition, fluency, executive functions and memory) and ALS show great overlap. This finding further supports the existence of a disease continuum, with ALS and FTD on both extremes. Deficits of social cognition and memory occur frequently in both diseases, which indicate that cognitive impairment extends beyond executive dysfunction.

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P49 QUANTIFYING COGNITIVE AND BEHAVIORAL CHANGES IN ALS OVER THE COURSE OF THE DISEASE

BERGGREN K[1], MATHY P[2], GIBSON SB[3], BROMBERG MB[3]

[1]*Department of Physical Med and Rehab, University of Utah Hospital and Clinics, Salt Lake City, UT, USA,* [2]*Department of Comm Sci an Dis, University of Utah College of Health, Salt Lake City, UT, USA,* [3]*Department of Neurology, University of Utah School of Medicine, Salt Lake City, UT, USA*

Email address for correspondence: kiera.berggren@hsc.utah.edu

Keywords: frontotemporal dementia (FTD), cognitive, behavioural

Background: Up to 40% of patients with ALS develop cognitive symptoms consistent with frontotemporal dementia (FTD) or dysfunction. The ALS-CBS is a validated screening tool that assesses cognitive and behavioural performance. Prior studies have not examined scores over time to assess stability.

Objective: To explore the relationship of performance on the Amyotrophic Lateral Sclerosis Cognitive Behavioural Screen (ALS-CBS) over time.

Methods: There are two portions to the ALS-CBS: for the cognitive portion (completed by the ALS patient) a cut off score of $>16/20$ is considered cognitively normal, scores $<11/20$ are correlated with FTD, and scores between the two are suggestive of cognitive impairment; for the behavioural ratings (completed by the ALS caregiver) a cut off score of $>35/45$ is considered behaviourally normal, scores $<33/45$ are correlated with FTD, and scores between the two cut offs are highly suggestive of behavioural impairment.

71 patients were screened using the ALS-CBS (45 male, 63.4%; 26 female, 36.6%; average age 63.8 years). 19 of the 71 patients were screened twice, 6 + months apart (13 male, 68.4%; 6 female, 31.6%; average age 62.0 years).

Results: For the cognitive portion: 25.6% had scores in the normal range; 65.6% had scores suggestive of cognitive impairment; and 8.8% had scores indicating the likely presence of FTD. Among the 19 patients who completed the ALS-CBS twice, at the initial screen: 3/19 were in the normal range; 14/19 were in the cognitive impairment range; and 2/19 in the FTD range. At follow up: 7/19 were in the normal range; 10/19 in the cognitive impairment range; and 2/19 in the FTD range. Change in cognitive scores between timepoints ranged from -3 to $+4$ at follow up.

For behavioural ratings: 56% were in the normal range; 17.9% were in the range suggestive of behavioural impairment; and 26.2% were in the range associated with FTD behaviours. Among the 18 patients who had caregiver ratings twice: initial scores were 9/18 in normal range; 3/18 in the behavioural impairment range; and 6/18 in the FTD behavioural range. At follow up: 8/18 were in normal range; 5/18 in the behavioural impairment range; and 5/18 in the FTD behavioural range. Change in behavioural scores at follow up ranged from -27 to $+7$. There were no correlative factors between changes in cognitive scores and behavioural ratings between the two timepoints.

Discussion and conclusion: The ALS-CBS in our patient group suggests that ~75% have cognitive and ~44% have behavioural difficulties. There were discrepancies between patient performance and caregiver ratings. The levels of impairment are relatively stable over 6 months, however changes in both cognitive and behavioural scores in both directions, towards improvement or towards further disability, were noted. Overall, the ALS-CBS appears to be useful in measuring cognitive and behavioural changes over time.

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P50 A PILOT STUDY TO ESTABLISH RELIABLE TELEPHONE-BASED COGNITIVE TESTING FOR THE ALS PATIENT POPULATION

CHRISTODOULOU G[1], HUPF J[1], MURPHY J[2], WOOLLEY-LEVINE S[3], GOETZ R[4], GENNINGS C[5], FACTOR-LITVAK P[6], MITSUMOTO H[1]

[1]*Eleanor and Lou Gehrig MDA/ALS Research Center, New York, New York, USA,* [2]*Department of Neurology, University of California, San Francisco, California, USA,* [3]*Forbes Norris ALS Center, California Pacific Medical Center, San Francisco, California, USA,* [4]*New York State Psychiatric Institute and Department of Psychiatry, Columbia University, New York, USA,* [5]*Department of Biostatistics, Virginia Commonwealth University, Richmond, Virginia, USA,* [6]*Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, USA*

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

Keywords: frontotemporal dementia (FTD), cognitive testing, reliability

Background: A national, epidemiologic study performed entirely over the phone that examines the relationship between oxidative stress and sporadic amyotrophic lateral sclerosis (ALS) is currently underway. Since frontotemporal dementia (FTD) is a well-recognized co-morbid disorder with ALS, the necessity to evaluate FTD over the phone is integral.

Objectives: To establish a reliable battery of measures to assess cognitive functioning over the phone.

Methods: In order to assess the validity of an ALS-appropriate, telephone-based screening exam, subjects were administered both in-person and telephone-based testing and randomly assigned to reverse-order groups. The battery included a telephone-modified version of the UCSF Cognitive Test Battery for FTD: (1) The ALS Cognitive Behavioural Screen (ALS-CBS); 2) Written Verbal Fluency and Controlled Oral Word Association Test (COWAT); 3) the Frontal Behavioural Inventory (FBI-ALS); 4) Center for Neurologic Study-Lability Scale (CNS-LS), and 5) the Mini-Mental State Examination (MMSE) and Telephone Interview for Cognitive Status (TICS). ALS assessment consisted of the ALS Functional Rating Scale-Revised (ALS-FRS-R) and % Forced Vital Capacity (% FVC). Patients were assigned to two groups and first received in-person or telephone evaluations followed by the opposite mode of testing approximately two weeks later. Two interviewers, trained to administer the battery, performed the testing, with one interviewer carrying out both modes of testing for each patient.

Results: To date, 20 subjects have completed both over-the-phone and in-person testing. The mean age was 62 (+7.4), 40% of the samples were women, the mean ALSFRS-R score was 37.3 (+6.2) and the mean %FVC was 83.5 (+18.3). Reliability between telephone-based and in-person gold standard testing was assessed using intraclass correlations (ICC). Intraclass correlations ranged from 0.60 to greater than 0.74, indicating that telephone administration was comparable to in-person administration, as reliability was in the good-to-excellent range. Further, the mean scores of the tests did not differ when analyzed using paired t-tests.

Discussion and conclusion: The results of these analyses show that tests within this battery can be successfully used over the phone to assess cognitive functioning. We are continuing the pilot study to reach a total sample size of 30. The development of telephone-based cognitive testing has never been undertaken and could become an integral resource to population-based, research studies. This testing could be especially valuable for subjects with severe, progressive physical disability and high caregiver demands, such as ALS.

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P51 BEHAVIOURAL CHANGE IN PRIMARY LATERAL SCLEROSIS: A CASE SERIES

ELAMIN M[1], BEDE P[1], BURKE T[2], PINTO-GRAU M[2], PENDER N[2], HARDIMAN O[1,2]

[1]*Trinity Biomedical Sciences Institute, Dublin, Ireland,* [2]*Beaumont Hospital, Dublin, Ireland*

Email address for correspondence: marwaelamin08@gmail.com

Keywords: primary lateral sclerosis (PLS), behaviour, screening

Background: It is now well recognized that ALS patients can have co-morbid behavioural changes that range from subtle findings to severe impairment fulfilling the criteria for behavioural variant fronto-temporal lobar degeneration. However, the literature examining behaviour in other types of motor neuron disease such as primary lateral sclerosis (PLS) is very sparse.

Objective: We aimed to document the frequency and pattern of behavioural changes in PLS patients.

Methods: All patients attending a Tertiary Multidisciplinary Motor Neuron Disease Clinic underwent screening for behavioural changes using a specifically designed and recently validated behavioural scale. We identified and interrogated data pertaining to patients with primary lateral sclerosis. The rate of different behavioural changes in PLS patients was compared to that of 50 age, sex and education matched healthy control that were recruited during tool validation.

Comparisons were made using non-parametric tests for continuous variables and Chi square with Monte-Carlo correction for proportions.

Results: Seven PLS patients were identified**.** Mean age was 62.9 years and 5 were males. Median R-ALSFRS score was 39.0 and median disease duration at time of testing was 100 months.

Two out the seven patients had scores above the cut off for abnormal behaviour, in both cases in within the range for severe behavioural change (as opposed to mild). These two patients were the oldest patients in the series and had the lowest ALSFRS-R scores in the cohort.

The most frequently reported behavioural change in PLS patients was over-sensitivity to external stimuli such as touch, smell etc reported in 6/7 PLS patients compared to 1/50 controls ($p < 0.0001$). Other behavioural changes observed more frequently in PLS patient compared to controls were inability to plan or foresee/solve problems ($p < 0.0001$); aggressiveness ($p < 0.0001$); repetitive behaviour ($p = 0.005$); self-centredness ($p = 0.005$); increased grammar mistakes ($p = 0.033$); and reduced display of emotion ($p = 0.013$).

Discussion and conclusion: Behavioural changes are not uncommon in PLS patients. Age and severe disease may be a risk factor. Larger studies are needed to confirm these findings.

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P52 COGNITIVE PHENOTYPES IN EUROPE

ABRAHAMS S[1], BAK T[1], NIVEN E[1], NEWTON J[1], PENDER N[2], HARDIMAN O[2], LULÉ D[3], LUDOLPH AC[3], VAN DEN BERG LH[4], POLETTI B[5], SILANI V[5], CHIÒ A[6], MORA PARDINA J[7], BURKHARDT C[8], WEBER M[8]

[1]*University of Edinburgh, Edinburgh, UK,* [2]*Trinity College, Dublin, Ireland,* [3]*University of Ulm, Ulm, Germany,* [4]*University Meidcal Centre, Utrecht, The Netherlands,* [5]*University of Milan, Milan, Italy,* [6]*University of Turin, Turin, Italy,* [7]*Hospital Caros III, Madrid, Spain,* [8]*Kantonsspital, St Gallen, Switzerland*

Email address for correspondence: s.abrahams@ed.ac.uk

Keywords: cognition, behaviour, phenotype

Objectives: The aim of this study was to develop and apply a single method for measuring cognitive change across European centres in an attempt to harmonize cognitive screening and compare cognitive phenotypes.

Methods: The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) has been previously shown to be sensitive to cognitive impairment in ALS (1). Here the ECAS was translated and applied to a total of 466 ALS patients (Scotland, n = 78, Ireland, n = 73, Italy-Milan n = 59, Italy-Turin n = 33, Holland, n = 40, Spain n = 40, Germany n = 73 and Switzerland, n = 35). Furthermore local normative data was collected within each country and cut-offs for abnormality determined.

Results: The ECAS was found to be sensitive to the types of impairment typically present in ALS patients across countries. The frequency of impairment within each country significantly differed ranging from 10 to 53% with a higher proportion of impaired patients from the samples in Ireland, Italy and Scotland and lower rates in Germany and the Netherlands. Furthermore the profile of impairment across cognitive domains also differed between countries with a greater proportion of patients with non-specific cognitive dysfunction (Memory, Visuospatial) in Ireland, while those in Scotland, Italy and Germany showed a more typical disproportionate impairment in ALS Specific functions (Executive, Language and Fluency).

There was a significant difference between samples in diagnostic delay and this significantly correlated with the degree of impairment in executive functions. Patients with a shorter delay indicating a more aggressive disease having more cognitive impairment. Furthermore duration of illness significantly correlated with ECAS scores, those patients with a longer duration of illness less likely to experience cognitive change. Age of onset also significantly correlated with cognitive impairment, with younger patients less likely to experience cognitive change. Frequency of impairment did not significantly differ according to symptom site of onset, which contradicts previously described associations with bulbar dysfunction, once tests which accommodate for speech and limb disability as the ECAS are used.

Genetic analyses were available from 81 patients. Those positive for the *C9orf72* mutation displayed ALS-specific cognitive dysfunction but were also more likely to have visuospatial dysfunction than those found to be negative for this mutation. The findings are related to clinical profiles including severity of disease and population differences.

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P53 PROGRESSION OVER TIME OF GENDER DIFFERENCES IN EMERGING ALS COGNITIVE DECLINE FROM THE TEMPORAL TO FRONTOTEMPORAL REGIONS

FLAHERTY C[1], BROTHERS A[2], VANKAMPEN D[3], SADEGHI A[1], YANG C[1], LEGRO R[1], MANNI A[1], SIMMONS Z[1]

[1]*Penn State College of Medicine, Hershey, PA, USA,* [2]*University of Colorado, Fort Collins, CO, USA,* [3]*Penn State University, Middletown, PA, USA*

Email address for correspondence: cflahertycraig@hmc.psu.edu

Keywords: gender, cognition, disease progression

Background: ALS is associated with frontotemporal lobar degeneration in ~50% of patients, in the absence of dementia, characterized by primary progressive aphasia and/or behavioural decline.

Objectives: We sought to investigate gender differences in pattern of emergence of cognitive decline in ALS and progression over 30 months, with repeat cognitive assessment every 6 months.

Methods: Beginning with a sample size of 356, we evaluated 152, 77, 48, 21 and 15 patients with the Penn State Brief Exam of Frontal and Temporal Dysfunction Syndromes (PSFTS) over the 30 month time period.

Results: Significant relative difficulties at time 1 were evident for females for 2-D constructions ($p = 0.044$), similarities ($p = 0.005$), judgment ($p = 0.018$), and calculations ($p = 0.045$). Medication records review of female oestrogen status for patients aged 31–74, showed a strong positive relationship between oestrogen levels and executive functioning capacities (similarities ($p = 0.005$), judgment ($p = 0.018$), letter fluency ($p = 0.004$)). At time 2, relative difficulties for females continued to be evident for 2-D constructions and calculations ($p = 0.15$), with a trend emerging for fluency advantage ($p = 0.110$). Time 1–2 comparisons evidenced progression among ALS cognitively normal (n = 80) to ALS cognitive impairment (ALSci) of 7.5%, with 25% now evidencing 1 deficiency and 67.5% remaining free of deficiencies. Gender differences were no longer evident by time 3, with 90% of patients found to be within the normal range on all measures administered. All patients remaining for times 4–6 were cognitively normal.

Discussion and conclusion: Gender differences are present in emerging ALSci, and relate to oestrogen status. Initial relative declines for females associate with the bilateral temporal regions subserving visual perception and verbal classification abilities, with a trend for relative difficulty for frontal cortical mediated working memory. With disease progression, female working memory relative disadvantage remains evident, while a female advantage begins to emerge for frontal cortical mediated verbal fluency. Time 1 findings evidence the potential of oestrogen as a therapeutic agent in emerging ALS-FTLD. Given the overlap in genes associated with ALS, FTLD and breast cancer, gonadal steroidal hormones likely serve as immuno-modulatory agents. Oestrogen analogs are needed to attenuate neurodegeneration while inhibiting over-activation in the breast and uterus, akin to the selective oestrogen receptor modulators currently applied as therapeutics in the treatment of breast cancer. Likely reasons for the lack of gender differences with disease progression include an increasing inability for individuals to pursue formal assessment due to ALS associated impairments, as well as the relative viability of individuals who remain alive and testable over 30 months, most of whom showed no progression of cognitive decline over time.

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P54 THE COGNITIVE RESERVE IN AMYOTROPHIC LATERAL SCLEROSIS WITH COGNITIVE IMPAIRMENT

CHIÒ A[1,2], PAGANI M[3], CISTARO A[4], MONTUSCHI A[1], IAZZOLINO B[1], BARULI MR[5], BARBERIS M[1,6], BRUNETTI M[1,6], RESTAGNO G[6], LOGROSCINO G[5], MOGLIA C[1], CALVO A[1,2], CANOSA A[1]

[1]*University of Turin, Turin, Italy,* [2]*Neuroscienze Institute of Torino, Turin, Italy,* [3]*CNR, Rome, Italy,* [4]*IRMET, Turin, Italy,* [5]*University of Bari, Bari, Italy,* [6]*AOU Città della Salute e della Scienza, Torino, Italy*

Email address for correspondence: achio@usa.net

Keywords: cognition, cognitive reserve, genetics

Background: 50% of ALS patients show variable degrees of cognitive impairment, ranging from frank frontotemporal dementia (ALS-FTD), to executive cognitive impairment (ALS-ECI), non-executive cognitive impairment (ALS-NECI) and pure behavioural impairment (ALS-Bi). A protective role of cognitive reserve (CR) has been reported in Alzheimer's disease (AD) and pure FTD. No data about ALS with comorbid FTD are available.

Objectives: To verify the CR hypothesis in ALS patients with different degrees of cognitive impairment.

Methods: 183 incident cases resident in Piemonte and diagnosed from January 1st 2009 to December 31st 2011 constituted our discovery cohort. Our validation cohort (n = 113) included incident cases from Piemonte between January 1 2012 and June 30 2013 and patients coming from other regions diagnosed in the same period. All patients underwent neuropsychological testing and genetic analysis. In the discovery cohort: 49.7% were cognitively normal; 12.6% ALS-FTD; 19.7% ALS-ECI; 5.5% ALS-NECI; 6.0% ALS-Bi; 6.0% non-classifiable cognitive impairment (ALS-NCCI); one AD. In the validation cohort: 50.5% were cognitively normal; 15.9% ALS-FTD; 18.6% ALS-ECI; 3.5% ALS-NECI; 6.2% ALS-Bi; 5.3% ALS-NCCI. A Reserve Index (RI) (2–12) was calculated from years of education and occupational attainment.

Results: In the discovery cohort ALS-FTD patients had lower education level (4.7 years, SD 1.9) than all other groups and lower RI (4.9, SD 1.3) than all other groups ($p = 0.0001$). In the validation cohort ALS-FTD patients (7.0 years, SD 2.6) had the same education level of ALS-NECI (7.0, SD 1.4), that was lower than all other cognitive categories ($p = 0.003$). ALS-FTD patients had lower RI (5.7, SD 1.6) than all other groups but ALS-NECI ($p = 0.003$). Results were independent from sex, age and site of onset and were confirmed among *c9orf72* expansion carriers ($p = 0.012$). Neuropsychological tests related to RI were TMT B, TMT B-A, Stroop Colour - Word Interference Test, WAIS-R Block Design, WMS-R-Form 2, FAB ($p = 0.0001$) and CPM total score ($p = 0.001$).

Discussion and conclusion: Reserve mechanisms may play a role in cognitive impairment related to ALS, primarily in full-blown FTD, including *c9orf72* mutations carriers. CR in ALS-FTD is mainly related to frontal functioning.

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P55 MIRROR NEURON FUNCTIONING IN ALS – A LINK BETWEEN MOTOR AND THEORY OF MIND IMPAIRMENT?

FLANAGAN E[1], YEW B[1], TAN R[1], ABRAHAMS S[2], KIERNAN MC[3], HORNBERGER M[4,1]

[1]*Neurscience Research Australia, Sydney, Australia,* [2]*University of Edinburgh, Edinburgh, UK,* [3]*University of Sydney, Sydney, Australia,* [4]*University of Cambridge, Cambridge, UK*

Email address for correspondence: mh755@medschl.cam.ac.uk

Keywords: mirror neuron, fMRI, theory of mind

Background: Amytrophic lateral sclerosis (ALS) is well known for its motor impairment and associated motor cortical changes. There is also increasing evidence that ALS patients show theory of mind (ToM) impairments. ToM is defined as the ability to attribute mental states, such as intentions, to others in order to understand and predict their actions. Interestingly, ToM has been associated with motor action observation in monkeys and healthy humans, via the so-called mirror neuron network. The mirror neuron network comprises dorsal and ventral premotor cortices, as well as inferior frontal gyrus (IFG) and inferior parietal regions. ALS cortical impairment in premotor regions could therefore potentially explain their ToM deficits. However, to date no study has investigated mirror neuron functioning in ALS, which could explain the relationship between premotor cortical integrity and ToM in these patients.

Objective: To establish the mirror neuron neural correlates in ALS.

Methods: Forty participants (ALS n = 22; age-matched controls n = 18) underwent a functional MRI experiment, which consisted of different action observations. Action observations involved hand-object interactions by an actor as well as a control condition (no interaction), based on previous monkey and fMRI work. Participants were shown all the videos before scanning commenced and watched blocks of each clip while lying in the scanner. The task was passive, not requiring motor responses from participants. Debriefing assured that participants watched and paid attention to the clips.

Results: Controls showed the expected mirror neuron network, including bilateral premotor and parietal cortices as well as IFG, when contrasting action observation and control conditions. By contrast, ALS patients showed markedly reduced activations for the same mirror neuron regions for the same contrast. The most marked differences were observed in the right dorsal and ventral premotor cortex as well as IFG for the action observation condition.

Discussion and conclusion: We demonstrate for the first time mirror neuron functioning in ALS. Our findings clearly indicate reduced activation in the regions implicated in mirror neuron function in ALS compared to age-matched controls. This was particularly pronounced over the right premotor cortices and IFG. These results give the first indication that ToM deficits in ALS might be inherently linked to the motor cortical deficits seen in this disease. Future studies need to investigate whether these mirror neuron changes are predictive of ToM functioning in ALS and might also explain ToM disturbances in related diseases, such as frontotemporal dementia.

In conclusion, mirror neuron network activations are reduced in ALS compared to controls, which establishes for the first time a link between motor and ToM impairments in this disease.

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P56 PREVALENCE AND IMPACT OF ALS BEHAVIOURAL IMPAIRMENT ON PATIENT AND CAREGIVER OUTCOMES OVER TIME

CAGA J[1], HOGDEN A[4], HSIEH S[1], LESLIE F[2], DEVENNEY E[2], AHMED RM[2], SIMON N[1], MIOSHI E[3], KIERNAN MC[1,5]

[1]*Brain and Mind Research Institute, Camperdown, NSW, Australia,* [2]*Neuroscience Research Australia, Randwick, NSW, Australia,* [3]*School of Clinical Medicine, University of Cambridge, Cambridge, UK,* [4]*Centre for Clinical Governance Research, Australian Institute of Health Innovation, University of New South Wales*

Randwick, NSW, Australia, [5]Sydney Medical School, University of Sydney, Camperdown, NSW, Australia

Email address for correspondence: jashelle.caga@sydney.edu.au

Keywords: behavioural impairment, depression, caregiver burden

Background: Frontotemporal syndromes more common than frank FTD, particularly mild behavioural and personality changes are associated with significant caregiver burden in ALS. Few studies have examined frontotemporal behavioural syndromes longitudinally and their implications for both caregiver and patient psychological wellbeing.

Objectives: To determine whether the prevalence of ALS behavioural impairment increases as physical functioning deteriorates and to determine the impact of ALS behavioural impairment on patient and caregiver mood and burden of care over time.

Methods: Sixty-one patients with ALS (53% male; mean age = 62.6 years, SD = 12.4 years) and their caregivers (38% male; mean age = 60.0 years, SD = 12.2 years) were included. Standardised measures were used to evaluate changes in behaviour, depression, caregiver burden and physical functioning over time. Patients were sub-classified into ALS pure or ALS with behavioural impairment (ALSbi) according to current consensus criteria for the diagnosis of frontotemporal behavioural syndromes in ALS.

Results: The average time interval for the follow-up assessment was 11.7 months (SD = 6.3). There was no significant change in the proportion of patients with behavioural impairment at baseline (29%) and follow-up assessment (36%), $p = 0.6$. ALSbi patients had significantly more depressive symptoms than patients without behavioural impairment at baseline only ($p < 0.05$). Burden of care in caregivers of patients with behavioural impairment was significantly higher compared to caregivers of ALS pure patients at baseline only ($p < 0.05$). There was no significant difference in depression levels of ALS pure and ALSbi caregivers at baseline ($p = 0.4$) and follow-up ($p = 0.8$). Patient ($p = 0.9$) and caregiver ($p = 0.6$) depression as well as caregiver burden ($p = 0.2$) did not change over time despite a significant decline in patients' physical functioning ($p < 0.05$).

Discussion and conclusion: The prevalence of ALS behavioural impairment did not increase as the disease progressed. Although patients with behavioural impairment had more depressive symptoms and their caregivers a greater sense of burden initially, patient and caregiver depression levels as well as caregiver burden remained stable over time. These findings suggest the importance of considering individual differences related to psychological and coping responses of ALS patients. This will provide much needed guidance on how patients are most likely to benefit from psychological support appropriate to their psychological status and needs.

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P57 THE RELATIVE IMPACT OF PATIENTS' DISEASE SYMPTOMS, COGNITION AND BEHAVIOUR ON THE PSYCHOSOCIAL WELLBEING OF ALS CAREGIVERS

WATERMEYER TJ[1,2], BROWN RG[1], SIDLE K[3], OLIVER D[4], KARLSSON J[5], ALLEN CE[6], SHAW CE[1,2], ELLIS CM[2], AL-CHALABI A[1,2], GOLDSTEIN LH[1]

[1]*King's College London, Institute of Psychiatry, London, UK,* [2]*King's College Hospital, Motor Nerve Clinic, London, UK,* [3]*Motor Neurone Disease Research and Care Centre, University College London Hospitals, London, UK,* [4]*The Wisdom Hospital, Kent, UK,* [5]*The Motor Neurone Disease Clinic, East Kent University Hospitals, Kent, UK,* [6]*The Motor Neurone Disease Clinic, Cambridge University Hospitals, Cambridge, UK*

Email address for correspondence: laura.goldstein@kcl.ac.uk

Keywords: caregiver outcomes, disease severity, cognitive-behavioural impairment

Background: In addition to the debilitating physical impairment of ALS, up to 50% of non-demented patients may experience mild-to-moderate cognitive and behavioural symptoms qualitatively similar to that shown by patients with Frontotemporal dementia (1). ALS patients have demonstrated deficits on tasks assessing executive function and social cognitive abilities, such as the ability to identify emotions from faces or attribute mental states to others (2). The relative impact of the physical and behavioural consequences of ALS on caregivers has been considered (3, 4), but no studies have examined if objective indices of patients' neuropsychological performance on tasks of executive function and social cognition contribute to caregivers' experiences of the disease.

Objectives: This study sought to examine the relative impact of patients' disease symptoms, behavioural involvement, executive dysfunction and impairment in social cognition on caregivers' psychosocial functioning.

Methods: Thirty-five spouse caregivers rated their mood, perceived level of burden and marital satisfaction. Spouses also rated their partners' behaviour in terms of everyday executive dysfunction, apathy, disinhibition and emotional lability. Composite scores of patients' performance on a battery of neuropsychological tests were created to measure their executive function and social cognition abilities. Patients' disease severity was also recorded.

Results: Bivariate correlational analysis was used to identify potential predictors of the caregiver outcomes. Regression analyses found that, together, the severity of patients' limb involvement ($\beta = 0.69$, $t(28) = 9.3$, $p < 0.001$) and behavioural problems ($\beta = -0.51$, $t(28) = -0.68$, $p < 0.001$) predicted caregiver burden ($R^2 = 0.84$, $F(2, 28) = 80.6$, $p < 0.001$). In addition, while limb symptom severity ($\beta = -0.45$, $t(31) = -2.8$, $p = 0.008$) predicted caregiver depression ($R^2 = 0.21$, $F(1,31) = 8.1$, $p = 0.008$), behavioural involvement ($\beta = 0.40$, $t(30) = 2.4$, $p = 0.0$) predicted caregiver anxiety ($R^2 = 0.16$, $F(1,30) = 5.65$, $p = 0.02$). Together, behavioural problems ($\beta = -0.42$, $t(27) = -4.65$, $p < 0.001$) and caregivers' self-rated marital satisfaction prior to their spouses' ALS ($\beta = 0.68$, $t(27) = 7.59$, $p < 0.001$) predicted caregivers' current marital satisfaction ($R^2 = 0.796$, $F(2,27) = 52.68$, $p < 0.001$). The cognitive composite scores were not associated with caregiver outcomes.

Discussion and conclusion: The study highlights the influence of ALS functional disability and perceived everyday behavioural dysfunction in ALS patients on caregivers' psychosocial health. The results suggest a possible specificity with which different ALS symptoms may impact on spouse caregivers. Caregivers' perceptions of cognitive-behavioural impairment in ALS patients may be more important for caregivers' outcomes than patients' objective neuropsychological performance. Clinical communication with ALS families should emphasize both physical and psychological challenges presented by the disease.

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P58 DEPRESSION IN PATIENTS WITH ALS/MND AND ITS ASSOCIATION WITH FUNCTIONAL STATUS AND COPING STRATEGIES

BONGIOANNI P[1,2], TRAMONTI F[2], FORTE M[1,2], VALORI F[1,2], ROSSI B[1]

[1]*Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy,* [2]*NeuroCare onlus, Pisa, Italy*

Email address for correspondence: bongioanni.paolo@gmail.com

Keywords: depression, coping strategies, functional status

Background: Data on depression in patients with ALS/MND show mixed results (1–2) and further research is needed to estimate the prevalence of mood disorders in such a population and better understand the relationship between depressive symptoms and other relevant variables.

Objectives: The aims of the present study are those of evaluating depressive symptoms in a population of patients with ALS and examining the associations between depression and functional status or coping strategies.

Methods: Scales for the evaluation of depression (BDI-II) (3), coping strategies (MND Coping Scale) (4) and functional status (ALSFRS) (5) were administered to 43 patients with ALS/MND. Bivariate correlations between the scales were calculated.

Results: The average BDI-II score is 16.17 ± 9.63. Most of the patients (approximately 70%) show minimal or mild depressive symptoms. Depression negatively correlates ($p < 0.05$) with functional status and with the following coping strategies: Positive Action ($p < 0.01$); Independence ($p < 0.01$); Positive Thought ($p < 0.01$).

Discussion and conclusion: Even if possibly biased by the items referring to physical symptoms of depression, the BDI-II scores of the present sample confirm that clinical depression is not widespread in patients with ALS/MND, and that perceived autonomy and active coping strategies could be related with better adjustment.

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P59 COPING STRATEGIES AMONGST NEWLY DIAGNOSED ALS PATIENTS

JAKOBSSON LARSSON B, NYGREN I

Department of Neuroscience, Neurology, Uppsala University, Uppsala, Sweden

Email address for correspondence: birgitta.jakobsson.larsson@neuro.uu.se

Keywords: coping strategies, newly diagnosed, support

Background: Amyotrophic lateral sclerosis is a fatal disease with impact on both physical function and psychological well-being. Patients use different coping strategies to manage symptoms and disease progression, but there is scarce knowledge about coping in an early stage of the disease.

Objectives: The aim of this study was to prospectively identify coping strategies used by newly diagnosed ALS patients and whether they change over time. We also wanted to determine if there were a correlation between physical function, psychological well-being, age and gender and the use of different coping strategies.

Methods: Thirty three patients were included in the study. Coping strategies was measured using the MND coping scale (1) and psychological well-being was measured with the Hospital Anxiety and Depression Scale (2). The patients' physical function was estimated with the revised ALS Functional Rating Scale (3). The evaluation was made one to three months, and six months after diagnosis.

Results: At both time points, support and independence was the most commonly used strategies, whilst the most seldom used were avoidance/venting and information seeking. Patients < 64 years old used positive action more often than older patients. There was a positive correlation between positive action and the patients' physical function at time point 1. Psychological well-being was correlated with the use of different coping strategies.

Discussion and conclusion: Support, which probably involves different kind of aids, seems to help patients to be independent and to cope with disease progression. Patients' psychological well-being correlated with different coping strategies and the use of different strategies changed over time.

The knowledge about the variation in use, and the correlation between coping strategies and psychological well-being in an early stage of ALS is important in developing support for the patient during disease progression.

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P60 INFORMATION NEEDS AND INFORMATION SEEKING PREFERENCES OF ALS PATIENTS AND THEIR CARERS

ABDULLA S[1,2], VIELHABER S[1,3], MACHTS J[3], HEINZE H-J [1,4], DENGLER R[2], PETRI S[2]

[1]*Otto-von-Guericke University Magdeburg, Magdeburg, Germany,* [2]*Hannover Medical School, Hannover, Germany,* [3]*German Center for Neurodegenerative Diseases, Magdeburg, Germany,* [4]*Leibniz Institute for Neurobiology, Magdeburg, Germany*

Email address for correspondence: abdulla.susanne@mh-hannover.de

Keywords: information seeking, internet search, caregivers

Background: Information seeking behaviour has been investigated in relation to numerous aspects of disease. This is of particular relevance in view of a rapidly advancing health information technology such as the Internet. Many studies refer to patients with cancer or other chronic diseases and are not easily applicable to patients with ALS. Two studies investigated the information seeking behaviour in ALS and PLS, one in Italy and one in the United States (1, 2). These data cannot easily be transferred to ALS patients in other countries as Internet access and health related Internet use differs between countries, even within Europe.

Objectives: To investigate the information seeking behaviour in patients with ALS and their caregivers and their rating of the usefulness of different information sources in Germany.

Methods: Survey in 106 patients and 100 caregivers in two university ALS outpatient clinics.

Results: Before seeing a doctor, 28% of patients and 23% of caregivers had used other sources to find symptom-related information, mostly via the Internet. Although two-thirds were satisfied with the way of diagnosis disclosure, 88% of patients and 85% of caregivers searched for additional information, most often on the internet (patients 72%, caregivers 85%), followed by patient brochures (patients 58%, caregivers 66%). Internet, patient brochures and the German Neuromuscular Disease Society were rated most frequently as useful/very useful. Traditional print media and interpersonal contacts were also frequently used and most respondents relied on more than one source for information. Only few respondents used the Internet for exchange with other patients. Two-thirds wanted to discuss web contents with their physician.

Discussion and conclusion: Patients with ALS and their caregivers clearly have additional information needs. Besides traditional information sources the Internet is frequently used. Therefore, reliable and useful websites should be provided. The patients' and caregivers' need to discuss their findings with a physician should be acknowledged.

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P61 THE SUBJECTIVE FEELING OF AUTONOMY AND FAMILY BONDING – CONFLICTING PRIORITIES IN DECISIONS TO PROLONG OR SHORTEN LIFE IN ALS

BÖHM S[1], HERKOMMER V[1], FANGERAU H[2], LUDOLPH AC[1], LULÉ D[1]

[1]*Institute of Neurology,* [2]*Institute of the History, Philosophy and Ethics of Medicine, University of Ulm, Ulm, Germany*

Email address for correspondence: sarah.boehm@uni-ulm.de

Keywords: decision-making, autonomy, caregivers

Background: The patients with amyotrophic lateral sclerosis (ALS) are faced with different decisions in the course of the disease regarding life prolonging (non-invasive ventilation, mechanical long-term ventilation and percutaneous endoscopic gastrostomy) and life shortening treatments (Euthanasia and physician assisted suicide). Determinants of these decisions are multifactorial and only some have been defined so far. The aim of this study was to identify possible determinants of the decision making process such as feeling of autonomy and family bonding.

Methods: A total of 100 ALS-patients were interviewed with questionnaires concerning their decisions of life prolonging and life shortening treatments. Possible determinants were acquired such as subjective feeling of autonomy (autonomy as core value, independence as coping strategy) and family bonding (demographic data, shared decision making). Furthermore, determinants such as subjective quality of life, depression, strategies in coping, religiousness and various factors of cognition were measured. Logistic and linear regressions were used for statistical analysis of the identification of determinants on decision making. Additionally, qualitative in-depth interviews ($n = 10$) were performed and analysed by qualitative content analysis.

Results: Family bonding was a strong determinant of decisions to prolong life. The majority (93%) of the patients named the wishes of their caregivers as important for them and 79% declared that the opinion of their caregivers influences their decisions. Similarly, increasing number of children of the patients showed a significant impact on the decisions to prolong life ($p = 0.03$, $R^2 = 0.38$). Concurrently, the patients showed a strong need for autonomy which turned out to be a strong determinant of decisions to shorten life ($p = 0.04$, $R^2 = 0.51$). Furthermore, degree of depression ($p < 0.01$

$R^2 = 0.21$) and religiousness ($p = 0.02$, $R^2 = 0.23$) had a significant influence on fatal decision making. Cognitive impairments however had no impact on decisions (all $p > 0.05$).

Discussion and conclusion: The results demonstrate a distinct discrepancy between the patients need for autonomy and the influence of the patient's family bonding on their decisions. Patients that are more influenced by their need for autonomy decide towards life shortening treatments, whereas the patients that are influenced by their family ties tend to decide towards life prolonging treatments.

Among other determinants, conflicting issues of subjective feeling of autonomy and family bonding have to be considered by the multidisciplinary teams in counselling, treatment and therapy of ALS patients.

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P62 AN EXPLORATION OF BEREAVED FAMILY CARERS' ACCOUNTS OF THE END-OF-LIFE EXPERIENCE OF PEOPLE WITH MND

O'CONNOR M[1], BENTLEY B[1], EDIS R[2]

[1]*School of Psychology and Speech Pathology, Curtin University, Perth, Western Australia, Australia,* [2]*Royal Perth Hospital Shenton Park MND Clinic, Perth, Western Australia, Australia*

Email address for correspondence: m.oconnor@curtin.edu.au

Keywords: dignity therapy, interviews, caregivers

Background: Palliative care is traditionally provided mainly to cancer patients. Expansion of palliative care services into non-malignant conditions has led to improvements in symptom control, quality of life and caregiver burden for people with MND. However, there are still gaps for people with MND and their families when it comes to access to specialist palliative care services. The end of life can be especially difficult for people with MND and families as the terminal phase in MND is not predictable and care needs are typically high. When there are gaps or problems in the provision of care at the end of life for people with MND, the burden falls squarely on family carers who are often overburdened and already experiencing high levels of distress. Identifying where the gaps are and what are unmet needs will help provide best practice end of life care for all people with MND and will help to reduce the burden on family carers and MND health professionals.

Objectives: This qualitative study explored the accounts of bereaved family carers about their perceptions of the end-of-life experience of people with MND.

Methods: Semi-structured interviews were used to elicit accounts of the experience of the end of life and the death of the person with MND from 12 bereaved family carers who were bereaved between three and 15 months. A social constructionist approach was used to elicit people's own experiences as the study was exploratory and applied. The study focused on three main areas: 1) the health care services used in the last three months of life with a special focus on the last week of life, 2) the information provided about the end of life to families from health care providers, and 3) the family carer's view of the death experience. The semi-structured approach allowed for people to give other information they felt relevant. Thematic analysis of the transcribed interviews was conducted. A former MND family carer and four MND specialist health care providers associated with an MND Clinic served as the Project Advisory Group to provide input into all aspects of the study.

Results: Three key themes emerged: The provision of support; information seeking; and preparation and readiness for death. Sub-themes included who people received support from, unmet needs, what worked well and personal strategies for coping. Recommendations for enhancing support and providing best care at the end of life are discussed.

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P63 THE EXPERIENCE OF MEDITATION IN ALS: A QUALITATIVE STUDY ABOUT THE EFFICACY OF A MINDFULNESS MEDITATION PROTOCOL WITH ALS SUBJECTS AND CAREGIVERS

PAGNINI F[2], LUNETTA C[3], TAGLIAFERRI A[4], MARCONI A[3], FOSSATI F[3], GRAGNANO G[1], GATTO R[4], FABIANI V[4], ROSSI G[3], SANSONE V[3]

[1]*Department of Psychology, Catholic University of Milan, Milan, Italy,* [2]*Niguarda Ca' Granda Hospital, Milan, Italy,* [3]*Neuro Muscular Omnicentre (NEMO), Fondazione Serena Onlus, Milan, Italy,* [4]*Department of Psychology, University of Milano Bicocca, Milan, Italy*

Email address for correspondence: francesco.pagnini@unicatt.it

Keywords: quality of life, mindfulness, meditation

Background: Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease, clinically characterized by progressive weakness leading to death by respiratory insufficiency, usually within three years. Although the patient's intellect and personality usually remain unimpaired, as the disease progresses the patient develops a worsening disability and impairment of the speech, often resulting in social isolation and a high degree of psychological suffering. The combination of ALS with progressive dependence and immobility might elicit feelings of despair, depression and anxiety.

To date, some of the most promising clinical treatments for the reduction of distress are based on mindfulness meditation practices, in particular the Mindfulness-Based Stress Reduction (MBSR), developed by Jon Kabat-Zinn. This program has proven useful to reduce stress levels and promoting resilience.

Objectives: This study tests the hypothesis that stress reduction methods based on mindfulness meditation can attenuate and prevent anxiety and depression, relieving the distress of living with ALS and providing skills to manage stressful events connected with the illness.

Methods: Sixty-three subjects with ALS were assigned to the ALS-MBI protocol or to a control condition (usual care). The Mindfulness-based Intervention protocol is derived from

MBSR, whilst respecting clinical peculiarities of ALS: physical exercises were removed, with a higher focus on the cognitive and affective issues. Other exercises, taken from different approaches with similarities to the mindfulness techniques (eg, Feldenkrais method), have also been inserted. Each of the eight sessions emphasized accepting the discomfort and physical limitation of ALS, focusing on resources and abilities that still remain. Seven exercises were devoted to mindful eating, breathing awareness, body scan, practice of loving kindness, Hatha yoga, music meditation, visualizations and motor imagery. Participants were asked to do homework on a daily basis. As part of a larger study, subjects were interviewed about their experience with mindfulness, aimed to understand the changes following the intervention. Qualitative data were analyzed with content analysis.

Results: Our preliminary results showed that both ALS patients and caregivers expressed positive evaluations on the mindfulness intervention. Acceptance and non-judgment seem to be powerful tools for the improvement of well-being in the ALS field. According to these preliminary results, the ALS-MBI protocol can improve (or restore, when required) the expression and the awareness of positive emotions.

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P64 DIGNITY THERAPY FOR PEOPLE WITH MOTOR NEURONE DISEASE AND THEIR FAMILY CAREGIVERS: A FEASIBILITY STUDY

AOUN S[1], CHOCHINOV H[2], KRISTJANSON L[3]

[1]*Curtin University, Perth, Australia,* [2]*Manitoba University, Winnipeg, Canada,* [3]*Swinburne University, Melbourne, Australia*

Email address for correspondence: s.aoun@curtin.edu.au

Keywords: dignity therapy, palliative care, caregivers

Background: There are calls to explore psychological interventions to reduce distress in Motor Neurone Disease (MND) patients and their family caregivers (FC). Dignity Therapy (DT) is a short term psychotherapy intervention shown to alleviate distress for people with life limiting illnesses. Patients are invited to discuss issues that matter most or that they would most want remembered about their life. Sessions are transcribed and edited with a final version (generativity document) returned to the patient, for the patient to bequeath to a family member or a friend, thus becoming part of a personal legacy.

Objectives: To assess the acceptability, feasibility and effectiveness of DT to reduce distress in people with MND and their FCs.

Methods: The study used a repeated measures design pre and post-intervention. Acceptability and feasibility were assessed using participants' ratings of the helpfulness of the intervention across several domains and time and resources required. Effectiveness measures for patients included: dignity-related distress (Patient Dignity Inventory); hopefulness (Herth Hope Index); spiritual wellbeing (FACIT-sp 12). Those for FCs included burden (Zarit Burden Interview); hopefulness (Herth Hope Index); anxiety; depression (HADS).

Results: 27 patients and 18 FCs completed the intervention. DT was well accepted including by patients who required assisted communication devices. The high satisfaction and endorsement of DT by patients suggests it has influenced various important aspects of end of life experience such as helped them attend to unfinished business and made them feel like they were still themselves. FCs overwhelmingly agreed that the DT document is and will continue to be a source of comfort to them and they would recommend DT to others in the same situation.

Discussion and conclusion: This is the first DT study to focus on MND and on home-based caregiving. The therapy needs to be offered earlier. Results established the importance of narrative and generatively for patients with MND and may open the door for other neurodegenerative conditions.

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P65 DO PATIENTS WITH MND EXPERIENCE DIFFICULTIES IN PERFORMING FINANCIAL ACTIVITIES AT HOME? EVIDENCE FROM A NOVEL ECOLOGICAL INSTRUMENTAL ADL TASK

MIOSHI E[1,5], BIER N[2], BOTTARI C[2], PRUD'HOMME M-P[2], CAGA J[3,6], RAMSEY E[3,6], HSIEH S[3,5], BAE J[4], DEVENNEY E[5,4], HODGES JR[5], KIERNAN MC[3,6]

[1]*University of Cambridge, Cambridge, UK,* [2]*Université de Montréal, Montreal, Canada,* [3]*Brain and Mind Institute, Sydney, Australia,* [4]*Hallym University, Seoul, Republic of Korea,* [5]*Neuroscience Research Australia, Sydney, Australia,* [6]*University of Sydney, Sydney, Australia*

Email address for correspondence: em341@medschl.cam.ac.uk

Keywords: functional disability, financial ability, neuropsychiatric symptoms

Background: With the recognition of the pervasive mild cognitive and neuropsychiatric symptoms in MND (1), it is critical to investigate how these symptoms may directly affect everyday living tasks requiring more complex reasoning.

Objectives: To investigate patients' ability to perform a well-validated ecological financial task.

Methods: 18 patients in the ALSFTD continuum (15 MND; 1 FTDMND; 2 FTD) were evaluated in an ecological finance task from the IADL Profile (2), well validated in patients with traumatic brain injury. The 'making a budget' task requires planning and management of expenses to achieve a fictitious goal. It assesses three operations: the ability to 1) plan; 2) carry out the task, including perceiving and correcting one's own errors during the task; and 3) verify if the task goal was successfully achieved or whether the task needs to be redone. Scores of independence are generated, one for each operation, based on type and amount of assistance required. Other assessments included global cognition (ACE-R: maximum score 100; cut off 88) and neuropsychiatric symptoms (MiND-B: maximum score 36; cut off 32).

Results: Only 22% of patients were completely independent in performing the IADL task. In fact, 61.1% of patients

required verbal assistance to complete the task and 16.7% could not complete the task at all. More specifically, patients had marked difficulty in 'carrying out the task' (83.3% were dependent), and difficulty in 'verifying attainment of goal' (77.8% required help). There was no clear difference in scores between the FTD, FTDMND and MND patients.

There was a strong association ($p < 0.05$; $R = 0.604$) between scores on the IADL task and the MiND-B, reflecting the relationship between ALS plus symptoms and difficulties in carrying out the budget task.

Discussion and conclusion: The great majority of patients in this study could not perform a complex ecological validated assessment of financial abilities independently or efficiently. These novel preliminary findings suggest that it may be advisable to oversee and support patients with neuropsychiatric symptoms as these can be affecting important daily functions.

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P66 AN ISOLATED DEFICIT: SOCIAL AFFECTIVE DEFICITS IN BULBAR ONSET ALS PATIENTS

BURKE T[1,2], PINTO-GRAU M[1,2], ELAMIN M[2], HARDIMAN O[1,2], PENDER N[1,2]

[1]*Beaumont Hospital, Dublin, Ireland,* [2]*Trinity College Dublin, Dublin, Ireland*

Email address for correspondence: burket2@tcd.ie

Keywords: neuropsychology, social-affective, impairment

Background: There is considerable debate about as to whether social cognitive processes are subsumed by metacognitive and executive functions, or whether they represent auxiliary frontotemporal processes. Cortical and subcortical atrophy has been noted in Amyotrophic Lateral Sclerosis (ALS), congruent with the neuroanatomical basis for social cognitive processes. The present study investigated Theory of Mind (ToM) deficits in patients with ALS.

Objectives: The aims of this research were to investigate whether social cognitive processes declined at a congruent rate to executive dysfunction in ALS.

Methods: Participants were recruited as part of an ongoing Irish population based study investigating cognitive decline in ALS. After removing patients whom were *C9orf72* positive, participants were grouped based on whether patients had bulbar ($n = 20$) or spinal onset ($n = 39$) ALS. Gender, age, IQ and education matched healthy controls were used to generate culturally specific comparative data for within-patient analyses ($n = 60$). Measures of social cognition included the Reading the Mind in the Eyes Test, measuring affective ToM and the Judgement of Preference Task, measuring cognitive ToM. Executive function was assessed using The Brixton Spatial Anticipation Test, Digit-Span and Lexical Fluency.

Results: On affective ToM, there was a significant difference between bulbar and spinal onset patients on this task ($p = 0.001$). Comparing bulbar and spinal patients standardized scores of executive function yielded no significant differences.

Discussion and conclusion: Results indicate the presence of social-affective deficits within ALS, prior to characteristic executive and language dysfunction, for bulbar onset patients without comorbid deficits. These scores may illustrate a decline in social cognition prior to other higher order functional deficits. Bulbar patients scored significantly lower than both spinal onset patients and controls, and this could be due to a number of contributory factors. These findings shall be discussed in relation to current neuroimaging research and neuropsychological theory.

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P67 EATING BEHAVIOR AND METABOLIC CHANGES ACROSS THE SPECTRUM OF MOTOR NEURON DISEASE AND FRONTOTEMPORAL DEMENTIA

AHMED RM[1,2], MACMILLAN M[1], MIOSHI E[1,3], CAGA J[1,2], IRISH M[1,2], BARTLEY L[1], HALLIDAY G[1,2], PIGUET O[1,2], HODGES JR[1,2], KIERNAN MC[4,1]

[1]*Neuroscience Research Australia, Sydney, Australia,* [2]*University of NSW, Sydney, Australia,* [3]*University of Cambridge, Cambridge, UK,* [4]*Brain Mind Research Institute, University of Sydney, Sydney, Australia*

Email address for correspondence: rebekahahmed@gmail.com

Keywords: frontotemporal dementia (FTD), metabolism, eating behaviours

Background: Increasingly it is recognised that motor neuron disease and frontotemporal dementia are spectrums of the one disease. Abnormal eating behaviours represent a core diagnostic feature for behavioural frontotemporal dementia (bvFTD), yet little is known about their effect on metabolic health and how this contrasts to the metabolic profile in motor neuron disease (MND).

Objective: We aimed to define the patterns of eating behaviour and intake in bvFTD and examine the association between eating behaviours and metabolic health of FTD compared with MND, where the metabolic profile has been hypothesised to affect disease progression. We hypothesised that as cognitive impairment increased across the spectrum of FTD MND so too would BMI and that changes in BMI, rather than being related to swallowing difficulties and loss of muscle mass may be centrally mediated.

Methods: Carers of 21 bvFTD, 26 Alzheimer disease control and 18 healthy control subjects completed validated questionnaires on appetite and eating behaviour. Body mass index (BMI), and blood samples measuring cholesterol and insulin levels were prospectively collected. BMI measurements were compared to a cohort of 100 MND (bulbar and limb) and FTD MND patients.

Results: BvFTD patients displayed significant abnormalities in all domains of eating compared to AD patients. BvFTD patients had significantly increased carbohydrate intake and a trend towards increased sugar intake compared to controls, yet they had similar hunger and satiety levels.

BMI measurements were significantly higher ($p < 0.01$) in the bvFTD, FTD MND and MND plus groups compared to the pure MND (bulbar and limb) and control groups. BvFTD patients had significantly ($p < .001$) increased insulin levels, triglyceride levels and an increased total cholesterol to HDL ratio, and a lower HDL cholesterol level compared to controls, suggesting they are insulin resistant and hyperlipidemic.

Discussion and conclusion: Abnormal eating behaviour is prominent in bvFTD, and is associated with increased sugar and carbohydrate intake and not explained by changes in satiety and hunger. Similar findings have been found in obese individuals and related to changes in the hypothalamus. Abnormal eating behaviour in FTD is associated with changes in BMI, and a blood metabolic profile similar to that seen in MND. BMI tends to increase as cognitive impairment develops in MND suggesting a FTD MND continuum, and that similar cortical structures to those involved in FTD may be involved in MND. The present findings have prognostic implications in terms of disease progression and may help to better delineate the metabolic profile of MND and FTD patients.

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THEME 4 RESPIRATORY AND NUTRITIONAL MANAGEMENT

P68 NUTRITIONAL SUPPORT IN AMYOTROPHIC LATERAL SCLEROSIS

IVANOVA M[1], BLINOV I[3], KOVALENOK K[3], BRYLEV L[2], AVDUNINA I[1], LYSOGORSKAYA E[1], CHERVYAKOV A[1], VOROBYEVA A[1], FOMINYKH V[1], SHTABNITSKIY V[3], SONKINA A[4], FOMINYKH M[3], BYALIK M[3], DIKHTER E[3], ZAKHAROVA M[1]

[1]*Research Center of Neurology RAMS, Moscow, Russia,* [2]*Moscow Clinical Hospital 12, Moscow, Russia,* [3]*Miloserdie Medical Center, Moscow, Russia,* [4]*Russian National Research Medical University, Moscow, Russia*

Email address for correspondence: fspsbl@gmail.com

Keywords: dysphagia, quality of life, percutaneous endoscopic gastrostomy (PEG)

Background: Pyramidal tract neuronal death in ALS leads to impairment of voluntary movements including swallowing and respiration. Dysphagia is associated with fatal complications such as aspiration pneumonia, undernutrition and dehydration. Optimization of nutritional support in ALS patients is necessary. Studies assessing quality of life in ALS patients with dysphagia and gastrostomy risks and benefits are lacking.

Objectives: To assess benefits and challenges of nutritional support especially gastrostomy in Russian ambulatory ALS patients.

Methods: Dysphagia in ALS patients was evaluated clinically and in some cases by video-radioscopy. Mild dysphagia was managed through diet modifications. Patients with severe dysphagia underwent gastrostomy. Patients or carers were interviewed, 30 patients with gastrostomy were questioned 1 year after gastrostomy.

Results: Percutaneous endoscopic gastrostomy (PEG) was performed in all patients with severe dysphagia except one (radiologic placement). Mean disease duration before gastrostomy was 849 days. Forced vital capacity (FVC) before gastrostomy was $<50\%$ in 20 % of patients. 4 patients had short-term fever that reduced without treatment after gastrostomy. One patient had an anterior abdominal wall abscess which required replacement of the gastrostomy tube. 28 of 30 patients had a benefit from gastrostomy. Most of patients had weight stabilization; taking meals caused fewer difficulties and was less time-consuming. Gastrostomy was more favourable than nasogastral tube feeding utilized in several patients before gastrostomy since it did not cause mucosal irritation and hypersecretion.

Discussion and conclusion: PEG improves the quality of life of ALS patients with severe dysphagia, rarely causes major or long-term complications and should be used more frequently.

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P69 GASTROSTOMY IN PATIENTS WITH ALS: MENTAL REPRESENTATIONS IN PATIENTS AND IN PHYSICIANS

PIETRA S[1], BELINGHER C[2], VERSCHUEREN A[2], ROUSSEAU M-C[1], POUGET J[2]

[1]*Hôpital San Salvadour (AP-HP), hyeres, France,* [2]*Reference centre for neuromuscular diseases and ALS, Hôpital de la Timone, marseille, France*

Email address for correspondence: annie.verschueren@ap-hm.fr

Keywords: mental representation, quality of life, percutaneous endoscopic gastrostomy (PEG)

Background: Enteral nutrition via gastrostomy is the standard method for ALS patients needing nutritional support and has to be discussed respecting the patient's social and cultural background and their peculiarities. If guidance exists, a big part of the deliberation is of the responsibility of the physicians according to their appreciation and judgment. From then on, the mental representations of gastrostomy in the patient and in the physician, as well as the mutual interactions can influence the decisions and the implementation of the device.

Objectives: The objectives of this qualitative research study are to establish the mental representations associated with the gastrostomy of the ALS patients and of the physicians involved in their care.

Methods: Twenty six ALS patients answered a semi-directive interview on gastrostomy. 26 physicians answered another semi-directive interview on the same theme. The interview grid consisted of 7 questions about the time when gastrostomy was discussed: the overall mental representations; the feeling when it had been discussed; the doubt or the acceptance towards the device; the arguments for the positive points and for the negative aspects of gastrostomy; and finally a summarized word of free association. Each interview was recorded and then retranscribed to allow data processing with analysis of contents and discourse analysis (Tropes software).

Results: Gastrostomy was noted in a predominantly positive way (22/26 for patients and 21/26 for physicians), with a recognition of its relevance and its interest which is upper to the constraints. For the most part patients, even if there was hesitation, stated that gastrostomy was considered as 'must be made', but as late as possible. Concerning the physicians, they put into words the doubt (16/26) and questioned the interest of gastrostomy and the risk-benefit assessment in advanced diseases (8/26). The notion of quality of life was present only in the discourse of the physicians. The timing of gastrostomy insertion was an important point in the concerns of the patients which is scheduled as late as possible. The feelings were strongly associated with the time and the progression of disease. Offering gastrostomy led to ambivalence for 60 % of

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the physicians, making nutritional assistance concomitantly a way of meaning the progression of disease.

Discussion and conclusion: The discussion about nutritional support by gastrostomy makes the recognition of technical relevance of the device coexist psychologically with the fears of confrontation with the disease progression. This is the case for patients but also for physicians. As any paradoxical contents, these probably generate a strain and influence the physician's recommendations, as much as the reception by the patient.

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P70 EXPERIENCE OF AN EARLY-IMPLANTATION PERCUTANEOUS ENDOSCOPIC GASTROSTOMY PROTOCOL ON ALS PATIENTS IN A MULTIDISCIPLINARY CLINIC

PAIPA AP, GOMEZ PR, POVEDANO MP, MARTINEZ YA

Hospital Universitari de Bellvitge, Barcelona, Spain

Email address for correspondence: andresjpaipa@hotmail.com

Keywords: percutaneous endoscopic gastrostomy (PEG), early use, protocol

Background: A positive effect of percutaneous endoscopic gastrostomy (PEG) has been described in ALS patients. Its systematic use is recommended by international guidelines. Since the year 2011 we have implemented a protocolized PEG education and offer it to our ALS patients in the context of a multidisciplinary clinic. We sought to evaluate the effect of such a protocol within our population.

Methods: Retrospective analysis of ALS patient survival before and after the implementation of an early-implantation PEG protocol.

Results: We analyzed a total of 140 patients. Mean age at symptom onset was 63 years. Mean diagnostic delay was 9 months (rank 1 to 25). 71 patients had a bulbar onset(38 female, 33 male) and 68 a spinal onset (28 female, 40 male); this difference was not significant ($p = 0.2$).

Time from symptom onset to PEG was significantly reduced since the protocol implementation (mean 19.7 vs 25 months, $p = 0.009$). There was no difference on age, use of non-invasive ventilation (58% vs 54%) use of riluzole or diagnostic delay, although patients on the intervention group had a non-significant tendency to an older age (66 vs. 62 years, p 0.87).

The control group had a predominance of bulbar onset patients (56.5%) while after the protocol implementation there was a trend toward more spinal onset patients receiving PEG (58.3%, $p = 0.77$).

Only one patient had a surgical infection which prompted removal and later reimplantation of the PEG. There were no other adverse events.

Discussion and conclusion: The early use of PEG is a safe treatment on ALS patients. We did not find an effect on survival. PEG use must be understood as a treatment destined to improve quality of life and patient well-being.

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P71 SAFETY OF PEG TUBE INSERTION IN PATIENTS WITH ALS USING PROPOFOL SEDATION IN AN OUTPATIENT SURGICAL CENTER

KITTRELL P[1], TOBON A[1], JACKSON C[1], FINCKE C[2], MOTAZEDI T[1]

[1]*UTHSCSA, San Antonio, TX, USA,* [2]*Methodist Healthcare System, San Antonio, TX, USA*

Email address for correspondence: kittrellp@uthscsa.edu

safety, percutaneous endoscopic gastrostomy (PEG), sedation

Background: Practice guidelines published by the American Academy of Neurology for the management of patients with ALS recommend the use of percutaneous endoscopic gastrostomy (PEG) for prolonging survival. ALS patients with weak respiratory muscles (demonstrated by a Forced Vital Capacity (FVC) $< 30\%$) have been considered at high risk for complications related with the procedure. Our goal is to demonstrate the safety profile, peri-procedural and post-interventional complications of PEG tube insertion performed in an outpatient surgical center.

Objectives: To review the authors' experience of PEG tube placement in patients with amyotrophic lateral sclerosis with different degrees of impaired respiratory function.

Methods: Retrospective review of medical records of patients treated at the University of Texas Health Science Center of San Antonio ALS clinic that were referred for PEG tube placement using propofol sedation at an outpatient surgical center.

Results: From 2011 until 2014, 61 patients had PEG placement under propofol sedation. The average age was 64 (range 31–84) with 31 males and 30 females. Patients were assigned to three groups based on FVC (FVC $< 30\%$ 9 subjects, 30–50% 26 and $> 50\%$ 24). Total propofol dose ranged from 60 to 500mg. Procedure duration (anesthesia time/surgery time) averaged 29/11 minutes (range 19–45/4–27). Patients currently on Bipap brought their own devices and used them in the intra and post-operative period as needed. No patients required invasive ventilatory support in the immediate post op period and there were no symptomatic cardiac arrhythmias or hypotension. Four patients developed perioperative complications: (1) death of unknown cause at home 24 hours after procedure (pre-op FVC 64%); (2) cellulitis at peg site 2 weeks post insertion; (3) abscess at peg site 10 days post insertion that required drainage and replacement of feeding tube; (4) Transient desaturation during surgery that stabilized but two days later had respiratory event and died in the hospital (pre-op FVC 43%). Mortality rate at 30 days was 4.9% (3 out of 61). All three patients had FVC $> 40\%$. All patients that developed complications also had significant comorbidities. There was no correlation between complications and FVC, BMI, disease duration or ALSFRS scale.

Conclusions: PEG tube placement with propofol sedation and Bipap support performed at a specialized outpatient surgical center can be achieved at a reasonable risk even in patients with very low FVC (less than 30%).

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P72 ENTERAL TUBE FEEDING AND SURVIVAL PATTERN OF 407 PATIENTS WITH MOTOR NEURONE DISEASE

CHHETRI SK[1,2], BRADLEY BF[2], MAJEED T[1], LEA RW[2]

[1]*Preston MND care and Research centre, Lancashire Teaching Hospitals NHS Foundation Trust, Preston, UK,* [2]*University of Central Lancashire, Preston, UK*

Email address for correspondence: suresh.chhetri@lthtr.nhs.uk

Key words: enteral feeding, nutrition, percutaneous endoscopic gastrostomy (PEG)

Background: Motor Neurone Disease (MND) patients with dysphagia and impaired nutritional status are usually offered enteral feeding (EF). The findings of prospective and retrospective studies investigating the impact of EF on survival have demonstrated little consensus (1, 2).

Objective: To assess the impact of enteral feeding on survival of patients with MND.

Methods: We conducted a retrospective review of the MND database and case notes of MND patients between 2005 and 2012. We identified cases that had undergone gastrostomy tube placement for EF. Statistical analyses of association between clinical manifestation, survival and gastrostomy tube insertion were evaluated using SPSS version 21. A forward stepwise cox regression was used to evaluate whether EF offers survival advantage. We also assessed survival in the bulbar and limb onset subgroups with and without EF using log-rank analysis.

Results: A total of 407 patients were identified. 345 cases with complete data were analysed of which 213 were limb onset, 130 bulbar onset and 2 respiratory onset. 93 patients (31 limb onset, 61 bulbar onset and 1 respiratory onset) received enteral feeding. After adjusting for effects of gender, onset age, onset site, age at diagnosis and riluzole treatment, EF was not associated with a statistically significant survival advantage ($\chi 2$ (1) = 1.96, p = 0.16). Log-Rank analysis revealed no significant difference in survival times between bulbar onset and limb onset illness, either with or without EF (Log-Rank $\chi 2$ (1) = 0.56, p = 0.45). Median (95% CI limits) survival times for limb onset MND with and without EF were 777 days (498.67-1055.13) and 715 days (620.47-809.53) respectively. Median survival times for bulbar onset with and without EF were 799 days (677.64-920.36) and 645 days (414.77-875.24) respectively.

Discussion and conclusion: There are no appropriately designed trials to inform decisions on nutritional management of MND patients and clinical practice is largely guided by expert clinical opinion and consensus. Our retrospective review did not find a survival advantage with enteral feeding. However the effect of enteral feeding on quality of life, an important objective of any management strategy, remains unknown. The retrospective nature of our study and lack of randomisation are potential limitations. A prospective study to evaluate impact of enteral feeding on survival and quality of life in this challenging clinical population would provide further evidence to inform and influence best clinical practice.

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P73 PERCUTANEOUS ENDOSCOPIC GASTROSTOMY (PEG) PLACEMENT INCREASES SURVIVAL IN AMYOTROPHIC LATERAL SCLEROSIS: TWO DECADES IN REVIEW

STERLING LE[1], PLOWMAN EK[2], SIMPSON EP[1], APPEL SH[1]

[1]*Houston Methodist Hospital, Houston, TX, USA,* [2]*University of South Florida, Tampa, FL, USA*
Email address for correspondence: LSterling@houstonmethodist.org

Keywords: percutaneous endoscopic gastrostomy (PEG), survival

Background: Percutaneous Endoscopic Gastrostomy (PEG) placement is utilized to maintain nutritional status in individuals with ALS. Current practice guidelines recommend PEG placement prior to forced vital capacity (FVC) falling below 50% (1). Few studies with small numbers have examined the impact of PEG placement in ALS patients; therefore the specific impact on survival remains unclear.

Objectives: To determine the impact of PEG placement on survival in a large cohort of ALS patients.

Methods: A retrospective database analysis was performed on consecutive cases of individuals diagnosed with probable/definite ALS in accordance with the most current El-Escorial Criteria (2, 3) and seen at the MDA/ALS Clinic at Houston Methodist Hospital from 1992–2013. Demographic data extracted included: age, gender, disease-onset type, PEG status, and time to death from symptom onset. Frequency counts and descriptives were performed to determine pertinent characteristics of individuals with ALS presenting to this facility over the past 20 years. Survival curves were calculated using the Kaplan-Meier method for patients choosing to undergo PEG placement and for those whom did not choose PEG using a logrank test. A series of ANOVAs and an ANCOVA were performed to investigate impact of PEG status, gender and disease-onset type on survival time with alpha set at 0.05.

Results: 2192 ALS patients were seen over the 21-year period. 58.9% were male and 41.1% female; 74.9% demonstrated spinal-onset and 25.1% bulbar-onset; and 533 (24.3%) underwent PEG placement while 1659 (75.7%) did not undergo PEG placement. Overall mean survival time from date of first symptom onset was 39.13 months (SD: 28.52). In this cohort, males demonstrated significantly longer survival then females (means: 41.18 vs. 36.26 months), F (1) = 8.71, $p < 0.001$ and spinal-onset patients lived longer then bulbar-onset ALS patients (means: 41.46 vs. 32.48 months), F (1) = 23.21, $p < 0.0001$. The presence of a PEG increased average survival time by 4 months (42.05 vs. 37.74 months) in the entire group of ALS patients, F (1) = 5.97, $p < 0.01$. When stratified by disease-type, PEG placement did not significantly impact survival time in bulbar-onset patients (PEG: 32.74 vs. no PEG: 32.22), however increased average survival time by 9.5 months in spinal-onset patients (PEG: 48.50 vs. no PEG: 39.02 months).

Discussion and conclusion: In this retrospective review of a large cohort of ALS patients we report that gender, disease-onset type and PEG tube utilization significantly impact survival. Specifically, males, spinal-onset disease

type and the presence of a PEG tube favourably impacted survival.

PEG placement represents an important intervention for maintaining nutritional status in individuals with ALS and extended survival by 9.5 months in this cohort of spinal-onset ALS patients.

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P74 EARLY PERFORMED PERCUTANEOUS ENDOSCOPIC GASTROSTOMY (PEG) IN ALS PATIENTS

HERRERA HV, VIRGILI CS, TURÓN SJ, POVEDANO MP, ANDRES MB, LAZO LA TORRE C

Bellvitge hospital, Barcelona, Spain

Email address for correspondence: clazolatorre@gmail.com

Keywords: percutaneous endoscopic gastrostomy (PEG), nutrition

Background: Malnutrition or progressive weight loss in the early stages of ALS has been reported as a predictor of poor survival prognosis. Aetiology is multifactorial, with the most important factor, dysphagia, being prevalent in 60% of patients with bulbar onset.

PEG should be performed in the disease stages with preserved respiratory function (forced vital capacity $>50\%$, and arterial carbon dioxide pressure <45 mmHg), to avoid early death after PEG and to maintain QOL with nutritional intervention through PEG. Although the appropriate amount of energy to be administered is yet to be established, high calorie diet is expected to be effective for potential improvement of survival. PEG is also necessary as a safe administration route of opioids for palliative and end-of-life care.

Objectives: To analyse the optimal timing of gastrostomy and correlate with other prognostic factors such as ponderal evolution and complications associated with gastrostomy.

Methods: A retrospective analysis was carried out in patients with ALS disease who attended the ALS Multidisciplinary Unit of a tertiary center in Barcelona, during the period from January 2012 to November 2013. Patients were selected who needed gastrostomy, and were compared with other variables such as age of onset, type of onset, time from needed nutritional support to support being provided or rejected, weight loss, and complications associated with gastrostomy.

Results: PEG was performed in 41 patients, 59% were men. 26% had spinal, 15% bulbar and 2% respiratory onset. The mean age in years was 64yrs ($\pm$ 11yrs). 71% required respiratory support by no invasive ventilation. Nutritional support in 93% and respiratory involvement in 7% were the principal indications.

Timing from diagnosis to require a gastrostomy was 11 months (IC 95%: 6–25). Timing from its indication to gastrostomy being performed was 3.1 months (IC 95% 1.5 – 12). The weight loss during this period, by percentage of lost weight, was 8% and the mean was 4.9kg (IC 95% -7.37kg to -2.5kg) ($p<0.05$).

Weight stabilization was observed in all patients after a month using nutritional support by gastrostomy. The mean weight increasing was 0.46kg (IC 95%: -1.09 to 2.01).

34% of complications associated with PEG were observed, 86% being minor and 14% major complications, 50% were associated with infection and 14% to an involuntary extraction.

Mean survival in days were 335 (IC 95%: 396-274) from time of PEG performing. Survival was not influenced by PEG complications.

Discussion and conclusion: Percentage of weight lost before PEG performing is quite high. Tendency to stabilisation after using the nutritional support was observed. This stability gives almost one year in survival in most patients. A long delay in PEG placement is associated with greater weight loss, and consequently with decreased survival.

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P75 CAUSES OF WEIGHT LOSS AFTER PEG PLACEMENT IN ALS PATIENTS

SCHRÖDER D[1], WIJNEN C[2]

[1]*het Roessingh, Enschede, The Netherlands,* [2]*Spierziekten Nederland, Baarn, The Netherlands*

Email address for correspondence: jcwijnen@kpnmail.nl

Keywords: weight loss, dietician, percutaneous endoscopic gastrostomy (PEG)

Background: After PEG placement weight loss continues although to a smaller extent than before PEG placement.

Objectives: Our objective was to investigate possible causes of ongoing weight loss despite dietetical interventions.

Methods: We retrospectively studied the records of patients discharged from rehabilitation or deceased between January 2010 and July 2013 in 7 ALS rehabilitation centres in the Netherlands. 59 patients were studied but 48 patients (22 men; mean age at diagnosis 63 years) were included because of incomplete data. 33 patients had a bulbar and 15 a spinal onset. All patients were followed for at least 6 months after PEG placement. Data up to 24 months after PEG placement were collected but only data up to 12 months could be analysed. Weight, estimated energy requirements and dietetical interventions were measured.

Results: The mean energy requirement when walking was 2007 kcal/24 h ($N=37$); the energy requirement was lower (1890 kcal/24 h) when sitting in a wheelchair ($N=18$) and was lowest for people ($N=10$) sitting in an electric wheelchair (1837 kcal/24 h). Mean weight loss 3 months after PEG placement was -1.5 kg ($N=48$) but varied between -8 kg or weight gain of $+3$ kg. Mean weight loss between 3–6 months was -0.8 kg ($N=35$), 6–9 months -1.0 kg ($N=21$), 9–12 months -0.7 kg ($N=13$). Average weight loss per person after four 3-month intervals (12 months after PEG placement) was not significantly different ($p=0.357$) between bulbar (-1.1 ± 2.1 kg) and spinal patients (-1.7 ± 2.4 kg). The mean number of dietetical interventions each 3 months was 3, in the first 3 months after PEG placement 3.6 which declined to 2.5 after 12 months. The most prevalent reported causes of dietetical interventions between 0–3 months were loss of muscle mass and illness (eg, pneumonia), 3–6 months loss of muscle mass, illness and intolerance of additional (enteral) nutrition, 6–9 months intolerance

of additional nutrition and loss of muscle mass. Non-invasive ventilation (NIV) (N = 27) did not influence changes in weight in comparison with patients without NIV.

Discussion and conclusion: We did not notice hypermetabolism in our cohort of patients. We expected stabilization of weight in patients with NIV but this did not occur. We also expected further weight loss after PEG placement, with a difference between spinal and bulbar patients. Nevertheless, large individual variations in weight changes, also in time, were seen. The dietician adjusted weight loss each consult by adding calories. It remains to be elucidated why patients could no longer tolerate additional (enteral) nutrition. Weight loss after PEG placement can be limited due to dietetical evaluations and interventions, at least once a month.

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P76 THE IMMEDIATE LOW PROFILE BUTTON GASTROSTOMY: PATIENTS PREFER IT AND WE SHOULD PROVIDE IT

ONDERS R[1,2], ELMO M[1], KAPLAN C[1], KATIRJI B[1,2]

[1]*University Hospitals Case Medical Center, Cleveland, Ohio, USA,* [2]*Case Western Reserve University School of Medicine, Cleveland, Ohio, USA*

Email address for correspondence: raymond.onders@uhhospitals.org

Keywords: percutaneous endoscopic gastrostomy (PEG), buttons, diaphragm pacing

Background: Thirty percent of ALS/MND patients present with bulbar onset and dysphagia is present in over 81% of advanced ALS/MND patients. Practice guidelines from the ANN recommend gastrostomy placement before the FVC falls below 50%. Only 43% of patients who were recommended a gastrostomy chose the therapy (1). Patients declined gastrostomy placement for the following reasons: belief their swallow was adequate, general disdain and lack of knowledge. The standard gastrostomy offered is the long external tube, the percutaneous endoscopic gastrostomy (PEG). The low profile gastrostomy tube (button) has been around for over 30 years. Safety of primary button gastrostomy insertion in adults has been demonstrated for over a decade.

Objectives: To determine ALS/MND patient preference of feeding tube style.

Methods: Subgroup analysis of all ALS/MND patients who were being evaluated for diaphragm pacing and were offered gastrostomy at a single institution. Patients were informed and offered a choice between standard PEG and button.

Results: Between 2011 and 2014, 112 patients were evaluated for diaphragm pacing and 88 did not have feeding tubes. 24 patients presented with PEG already in place. During evaluation, 47 patients had bulbar symptoms with 34 of them presenting without PEG. 32 of the patients without PEG also had FVC below 49% (22–49). 61 out of 63(97%) patients choosing gastrotomies chose the button and were safely and successfully placed. Two patients received standard PEG. One patient had a button replacement in first week from balloon rupture. After two months, two patients required conversion to standard PEG due to body habitus. Patients that chose the button ranged in age from 28 to 81 years with an average age of 57years. The average FVC was 57% (17%-110%). 19 patients choosing the button had a FVC above 60% and no bulbar symptoms. There is a 100% 6-month survival. The survival range to date is 6 months to 28 months with an average of 14.3 months.

Discussion and conclusion: When given a choice, patients overwhelmingly selected the immediate button-97%. All patients preferred the aesthetics of it. This sample showed direct button placement is safe and has a very low complication rate in ALS/MND patients. Button changes are easily performed. Also, they can be easily converted to a standard PEG if needed. A limiting factor in offering a button gastrostomy is abdominal size. Utilization requires the abdomen to be exposed and greater finger/hand dexterity to access compared to a standard PEG. One of our patients chose the standard PEG so he could perform his own feeds. Offering direct button placement could affect ALS/MND patients' acceptance of a feeding tube allowing for placement at a safer stage of their disease.

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P77 REDUCED LIP FORCE IN MND PATIENTS WITH WEIGHT LOSS WITHOUT DYSPHAGIA – A PILOT STUDY

BACKMAN P

University Hospital, Uppsala, Sweden

Email address for correspondence: petra.backman@akademiska.se

Keywords: lip force, weight loss, dysphagia

Background: Patients with motor neuron diseases (MND) often experience weight loss and malnutrition. The underlying causes of the weight loss are not fully understood. It can occur with or without clinical findings of dysphagia and is associated with shorter survival (1). Previous research indicates a correlation between reduced lip force and dysphagia in stroke patients, both with and without facial palsy (2). Since some of the similar muscles can be affected in MND patients and stroke patients there is a possibility that lip force is reduced in MND patients as well.

Objectives: The aim of this pilot study was to explore if MND patients with weight loss without clinical findings of dysphagia have a reduced lip force compared to healthy controls.

Methods: Seven patients with MND and 10 healthy controls matched for age participated in the pilot study. The patients with MND had an involuntary weight loss during the last year and showed no clinical signs of dysphagia or dysarthria. All patients completed sequential water swallowing of 100 ml of water within 10 seconds. Lip force was tested with an oral screen attached to a force dynamometer (Lutron, FG-5005). The oral screen was placed in the mouth between the lips and the teeth and the participants were instructed to hold the oral screen in their mouths for as long as possible by tightening the lips. When the participants no longer could hold the oral screen it came out of the mouth. The force dynamometer showed a peek value in Newton (N). Each participant made five attempts and the highest value of the peak values was registered.

Results: The results showed a reduced lip force in the patients with MND compared to the healthy controls. The MND patients mean lip force was 22.8 N (median 20.8; SD 6.1)

and the mean lip force in the healthy control group was 42.5 N (median 45.2; SD 8.9).

Discussion and conclusion: The results indicate that patients with MND may have a subclinical weakness in their lips even when there are no apparent signs of dysphagia or dysarthria. Given the poor prognosis associated with weight loss it may be valuable to discover difficulties as early as possible for appropriate disease management. Further research is needed to explore lip force and how it affects MND patients.

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P78 ANTHROPOMETRY OF ARM: NUTRITIONAL RISK INDICATOR IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

SALVIONI C, STANICH P, PAULI M, CHIEIA M, QUADROS A, OLIVEIRA A

Federal University of Sao Paulo, Sao Paulo, Brazil

Email address for correspondence: pstanich@uol.com.br

Keywords: nutritional assessment, nutrition, anthropometry

Background: Nutritional parameters of ALS patients worsen during disease. For nutritional evaluation the body mass index (BMI) instrument alone does not reflect changes across regions of the body.

Objectives: To correlate clinical, nutritional and respiratory data with functional parameters.

Methods: 111 ALS patients between 2009 and 2010 took part in the study. Clinical data collection consisted of: site of onset; time referred to disease (TRD); and date of diagnosis. Assessment times were divided into 3 groups: T1 (symptoms until diagnostic confirmation); T2 (symptoms and nutritional evaluation); T3 (diagnosis and nutritional evaluation). The anthropometric assessment included: triceps skinfold thickness (TSF); midarm circumference (MAC); arm muscle area (AMA); midarm muscle circumference (MAMC); and arm fat area (AFA). For classification of nutritional status, BMI and anthropometry were examined individually and later in association with protein-caloric malnutrition score (PCMS). ALSFRS scale was applied and analyzed individually and grouped in main domains. Each item in the scale was correlated with the second site of onset, spinal group (GA) or bulbar group (GB).

Results: In patients from the bulbar group (GB) the T1 and T3 was earlier with respect to those patients in the spinal onset group (GA). A statistically significant difference was observed for T2 ($p < 0.012$). On correlation between anthropometric parameters and times, to GA we observe that T2 showed significant positive correlation with %TSF and negative for MAMC, %MAMC, AMA and %AMA. For T3, positive significant association was found with TSF, %TSF, whilst a negative association was found with MAMC. The analysis between FVC and anthropometric measurements showed that there was a significant association for GA and GB. In GA there was also a positive correlation with MAC, MAMC, % MAMC, AMB and PCMS, while for GB only a correlation could be found with body weight. On correlation of nutritional parameters and ALSFRS for GA patients we observed that MAC and % MAC presented positive association with both issues of D1 and D2. For GB, total score: in addition to a positive correlation with anthropometric parameters related to lean body mass (MAMC, AMA and %AMA) there was also a negative association associated with body fat.

Discussion and conclusion: A positive association was found between T2 and TSF, negative with all measures of muscle mass estimate suggests that delay in nutritional intervention can have a negative effect on loss of muscle. With respect to FVC and anthropometric measures we can infer that respiratory loss can be a sign of nutritional decline, with the reverse also being true. On correlation between anthropometry and ALSFRS, the results suggest that evaluation of measures, in particular measures to estimate muscle mass, may be related to motor impairment in evolution disease.

Anthropometry of arm was important for analysis justifying the importance of nutritional evaluation. The ALSFRS, in particular the D2, indicated nutritional impairment. The application of scale could serve as screening instrument, in order to anticipate the referral to dietician working with better prognosis in ALS.

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P79 DOES BULBAR DYSFUNCTION RENDER RESPIRATORY ASSESSMENT BY STANDARD PULMONARY FUNCTION TESTS MEANINGLESS? QUANTIFYING THE IMPACT OF BULBAR DYSFUNCTION ON MEASURES OF RESPIRATORY INSUFFICIENCY IN 100 PATIENTS WITH ALS

JENKINS L[1], SAKAMURI S[1,2], MILLER RG[1], FORSHEW D[1], GUION L[1], MOORE D[1], KATZ J[1]

[1]*Forbes Norris ALS/MDA clinic, California Pacific Medical Center, San Francisco, California, USA,* [2]*Stanford Hospital & Clinics, Stanford, California, USA*

Email address for correspondence: liberty.jenkins@me.com

Keywords: respiratory, phrenic, bulbar

Background: Diaphragmatic weakness is the primary cause of hypoventilation in ALS. Phrenic nerve conduction studies assess diaphragmatic innervation and correlate with respiratory failure (1). In contrast, standard pulmonary function tests (PFTs), when used in ALS, are affected by other factors including accessory muscle weakness and bulbar dysfunction (2).

Objectives: To examine how bulbar dysfunction affects PFTs in ALS.

Methods: We prospectively recorded phrenic nerve conduction studies, upright and supine FVC, maximum inspiratory pressure (MIP), sniff nasal inspiratory pressure (SNIP), ALSFRS-R and bulbar subscore (FRSb) in 100 patients with ALS.

Results: Of the 100 subjects: 41 were female, 69 had spinal onset, and the average symptom duration was 24.2 months. The correlation co-efficients between diaphragmatic CMAP amplitude (Pamp) and standard PFTs were: supine FVC 0.48

($p < 0.001$); upright FVC 0.45 ($p < 0.001$); MIP 0.37 ($p < 0.001$) and SNIP 0.28 ($p < 0.001$)). To quantify the independent effect of bulbar dysfunction on these measures, we used logistic regression analysis to eliminate the joint effect of Pamp on the PFT results. We found a marked decline in the performance of all standard respiratory measures as a result of bulbar dysfunction alone, independent of diaphragmatic innervation ($p < 0.001$). The effect on MIP was substantial: a drop on the FRSb from just 12 to 11 resulted in a fall in the MIP from 65cmH_20 to 54cmH_20, and to 44cmH_20 at a score of 10. The SNIP was similarly affected. The bulbar related drop in FVC was less, falling from 88% predicted at an FRSb of 12, to 82% at 11, and to 75% at 10. More moderate bulbar involvement caused PFT results to decline even further into 'abnormal' ranges. For example, an FRSb score of 6 resulted in a MIP of 32cmH_20, a SNIP of 20 cmH_20 and an FVC of 60%, independent of diaphragmatic dysfunction as measured by phrenic nerve conduction studies.

Discussion and conclusion: Standard PFTs are of limited utility in the assessment of diaphragm dysfunction. The presence of modest bulbar disease leads to results so abnormal that the clinician is essentially blind to the true state of the diaphragm, while a drop of just one point on the FRSb resulted in predicted MIP values below the threshold for initiating NIV, presumably because of bulbar factors alone. FVC and MIP are still the main instruments used in respiratory assessment and clinical trials. We show that phrenic nerve conduction studies, or other direct assessments of the diaphragm, may be needed to provide key data on diaphragm dysfunction in bulbar patients.

Acknowledgements: We are grateful to our patients for contributing their time to this research.

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P80 ASSESSING DIAPHRAGM FUNCTION IN ALS: COULD A STANDARD INSPIRATION CHEST X-RAY WITH AN ADDITIONAL RELAXATION VIEW PROVIDE THE ANSWER?

ONDERS R[1,2], ELMO M[1], KAPLAN C[1], KATIRJI B[1,2], SCHILZR[1,2]

[1]*University Hospitals Case Medical Center, Cleveland, Ohio, USA,* [2]*Case Western Reserve University, Cleveland, Ohio, USA*

Email address for correspondence: raymond.onders@uhhospitals.org

Keywords: diaphragm pacing, chest x-ray, hypoventilation

Background: The diaphragm is the primary muscle of respiration and failure of function leads to hypoventilation and respiratory failure. Assessing diaphragm function in patients with ALS/MND involves functional pulmonary function tests but these tests may underestimate unilateral diaphragm dysfunction. Standard chest x-ray can have patients air stacking which can falsely suppress abnormal unilateral diaphragm. Fluoroscopic sniff tests assess bilateral diaphragm function but can be difficult to perform in all centres. Also the use of accessory respiratory muscles can give the false appearance of diaphragm movement.

Objective: This report analyses a new simplified radiographic technique of assessing ALS patients' upper motor neuron (UMN) control of their diaphragm.

Methods: Consecutive patients with ALS/MND at a single site under an IRB approved protocol to assess for suitability of diaphragm pacing were evaluated. All patients had a standard assessment which included: pulmonary function tests; arterial blood gasses; fluoroscopic sniff diaphragm analysis using a grid measuring system; phrenic nerve conduction studies (PNCS); and subsequent intra-operative diaphragm strength grading under direct stimulation, which identifies the intact lower motor neurons (LMN) with their associated diaphragm motor units. In addition each patient had a standard chest x-ray with the addition of a relaxation image after they take a breath. The inspiration compared to relaxation should correlate to the continuous fluoroscopic evaluation of the diaphragm.

Results: 26 consecutive ALS subjects had this battery of tests with the addition of inspiration/relaxation chest x-rays. 19 patients (73%) had diaphragm movement correlation between inspiration/relaxation diaphragm movement and fluoroscopy. In analysing each hemidiaphragm separately there was a 77% correlation (40 of 52). In a multivariate analysis of all factors the following relationships had a p-value < 0.05: forced vital capacity (FVC) is correlated to maximal inspiratory pressure (MIP) and radiographic diaphragm movement; MIP is correlated to radiographic diaphragm movement; and arterial CO_2 is correlated to radiographic diaphragm movement- in which hypercarbia is related to decreased movement of diaphragm. The operative assessment of diaphragm movement under direct stimulation did not correlate to pre-operative testing.

Discussion and conclusion: The simplified inspiration/relaxation chest x-ray correlates with fluoroscopy and can easily be performed radiologic departments. The inspiration/relaxation chest x-ray is 50% less expensive in charges in the US (461 versus 993 US dollars). These tests analyze UMN control of the diaphragm but diaphragm pacing is based on the intact LMNs. Patients with poor diaphragm movement on these radiologic tests can be good diaphragm pacing candidates if they have intact LMNs to stimulate as identified by PNCS or intraoperative assessment. PNCS primarily assess anterior diaphragm while posterior diaphragm is the most important diaphragm for ventilation. Laparoscopic diaphragm evaluation focuses on posterior diaphragm and the ability to stimulate the LMN motor units that have lost UMN control is how diaphragm pacing improves ventilation.

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P81 LONG TERM SURVIVAL OF ALS/MND PATIENTS WITH DIAPHRAGM PACING IMPLANTATION WITH LOW FORCED VITAL CAPACITY BELOW 45% PREDICTED

ONDES R[1,2], ELMO M[1], KAPLAN C[1], KATIRJI B[1,2], SCHILZ R[1,2]

[1]*University Hospitals Case Medical Center, Cleveland, Ohio, USA,* [2]*Case Western Reserve University School of Medicine, Cleveland, Ohio, USA*

Email address for correspondence: raymond.onders@uhhospitals.org

Keywords: diaphragm pacing, respiratory failure, forced vital capacity (FVC)

Background: Forced vital Capacity (FVC) is widely used as an indicator of prognosis in ALS, need for non-invasive ventilation (NIV) or diaphragm pacing (DP). Therapy with DP may be excluded because of no previous data or experience if the FVC is less than 45% predicted. Previously it has been described that there can be adequate stimulatable intact motor units with associated lower motor neurons in ALS patients with FVC below 45% predicted. (1). This reports the long term survival of patients implanted with diaphragm pacing with a FVC below 45% predicted.

Objective: To analyze surgical morbidity, mortality and long term survival patients with FVC < 45% implanted with DP.

Methods: Retrospective analysis of all patients implanted with DP and a FVC below 45% predicted from a prospective database at a single site under an IRB protocol for ALS patients from November 2011 until April 2013.

Results: 24 ALS subjects were implanted with FVC below 45%. The average FVC at implant was 35.6 +/−7.9 (range 17–45); average MIP was 26.4 +/−9.7; and PCO_2 was 45.0 +/−7.3. There was no peri-operative mortality with only one early tracheostomy although DP was continued. Within the first 6 months, two other early deaths involved uncontrollable secretions and an additional one involved a mucous plug. Only one patient stopped DP within two months of surgery and has been lost to long term follow-up. The median survival using Kaplan Meier analysis is 11.8 +/−2.9 months. 9 out of the 24(37.5%) patients are still alive an average of 19.33 months post implant with the longest at 29 months.

Discussion and conclusion: DP surgery can be safely implanted in patients with FVC below 45%. In this late stage group of patients there is still a stimulatable diaphragm motor units were DP can delay respiratory failure and subsequent death with a significant patients surviving over 19.3 months post-surgery. Patients should be adequately informed about options to maintain diaphragm function with DP even when late in the disease course.

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P82 EFFECT OF TIRASEMTIV ON SUBMAXIMAL RODENT DIAPHRAGM STRENGTH AND RESPIRATORY FUNCTION

HWEE DT, KENNEDY A, MALIK FI, JASPER JR

Cytokinetics, Inc., South San Francisco, CA, USA

Email address for correspondence: dhwee@cytokinetics.com

Keywords: diaphragm, respiratory function, calcium sensitivity

Background: Diaphragm weakness, which is characterized by significant losses in function, is a primary component of the pathophysiological changes that lead to respiratory failure. *Tirasemtiv* is a fast skeletal troponin activator, previously shown to increase submaximal force in rat and human lower leg muscles.

Objective: The objective of this study was to characterize the effect of *Tirasemtiv* on calcium sensitivity and force production *ex vivo* in rat and mice diaphragm muscle.

Methods: For *in vitro* skinned (permeabilized) fiber studies, Sprague Dawley rat diaphragm muscles were rapidly dissected, rinsed in physiological saline, and then incubated in skinning and storage solution. Single muscle fibers were dissected from larger segments of tissue in rigor buffer. The fibers were then suspended between a force transducer and a fixed post. The muscle force-calcium (pCa) relationship in diaphragm muscle was investigated in single rat diaphragm fibers treated with either 1% DMSO (vehicle treatment), or *Tirasemtiv* (0.1μM, 1μM, or 10μM) over –log (10) calcium concentrations (pCa) ranging from 8 to 4.

For intact diaphragm muscle, contractile force was measured by electrical field stimulation in an organ bath system. The diaphragm and the last floating rib from B6SJL mice were excised, rinsed in physiological saline, placed in a temperature controlled water-jacketed chamber containing Krebs-Henseleit buffer. Braided silk sutures were tied at the central tendon and floating rib and attached to a force transducer between two platinum electrodes. The force-frequency profile of the muscle was obtained by stimulating the muscle at frequencies between 5–150 Hz. *Tirasemtiv (*1 μM in DMSO) was directly added into the bath.

Results: *Tirasemtiv* increased the calcium sensitivity of rat diaphragm muscle, shifting the force-pCa relationship of skinned fibers in a dose-dependent manner. Compared to DMSO-treated skinned diaphragm muscle fibers, 10μM *Tirasemtiv* increased the pCa at 50% maximum tension (pCa_{50}) 10-fold (vehicle: 5.43 ± 0.05, 10μM *Tirasemtiv*: 6.74 ± 0.02, n = 5/group). In intact muscle, at submaximal stimulation frequencies less than 20Hz, *Tirasemtiv* (1μM) increased mouse diaphragm tension *ex vivo* compared to vehicle-treated diaphragm strips (vehicle n = 11,T*irasemtiv* n = 5, $p < 0.05$ at 5, 10, and 20 Hz).

Discussion and conclusion: Pathological conditions that lead to diaphragm weakness can have severe consequences, ranging from dyspnea and reduced quality of life to respiratory failure and death. The fast skeletal troponin activator, *Tirasemtiv,* increased calcium sensitivity in a dose-dependent manner and increased submaximal force production *ex vivo*. These results suggest that *Tirasemtiv* and other fast skeletal muscle troponin activators may be viable therapeutics for improving respiratory muscle function.

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P83 LONG-TERM IMPACT OF EXPIRATORY MUSCLE STRENGTH TRAINING ON RESPIRATORY FUNCTION AND DISEASE PROGRESSION IN TWO PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS: A CASE SERIES

ROSADO KM, TABOR L, TURNER K, GAZIANO J, RICHTER J, VU T, GOOCH C, PLOWMAN EK

University of South Florida, Tampa, Florida, USA

Email address for correspondence: plowman@usf.edu

Keywords: Expiratory Muscle Strength Training (EMST), ALSFRS-R, exercise

Background: The role of exercise in individuals with ALS is controversial. We have recently reported that expiratory muscle strength training (EMST) is feasible, safe and leads to improvements in expiratory force generating pressures immediately following a five-week treatment program in a pilot

study of 25 ALS patients. The long-term impact of EMST on respiratory function and disease progression in this patient population has not yet been studied.

Objective: To determine the long-term impact of expiratory muscle strength training on expiratory force generating pressures (maximum expiratory pressure) and global disease progression (ALSFRS-R) in two individuals with ALS.

Methods: Two individuals with a diagnosis of ALS (Revised El-Escorial Criteria) participated in an eight-week EMST program. Training consisted of 5 weekly sessions of 25 repetitions at 50% of individualized MEP. Outcome measures included MEPs and ALSFRS-R scores, evaluated at baseline, immediately post-EMST, and every three-month post-EMST.

Results: Patient 1 was a spinal-onset 58-year-old male, 11 months post-symptom onset and a Baseline ALSFRS-R score of 45. Following eight-weeks of EMST, he demonstrated a 43% improvement in MEPS (179.66 vs. 257.00cmH_20) and his ALSFRS-R score remained unchanged. At this time point, Patient 1 continued the EMST program. At the three- and six-month follow-up evaluations, Patient 1 demonstrated a 26.71% and 14.47% improvement of MEPs respectively compared to his baseline MEP (227.66cmH_20 and 205.66 cmH_20). His ALSFRS-R did not decline across the three- and six- month time points.

Patient 2 was a 67-year-old bulbar-onset male, 14 months post-symptom onset and a Baseline ALSFRS-R score of 37. Following eight-weeks of EMST, MEPS increased by 41% (104.00 vs. 146.67cmH_20) and his ALSFRS-R score did not change. Patient 2 ceased EMST at this time point. At the three- and six-month follow up evaluations, he demonstrated a 36.22% and 58.01% decline in MEPs from baseline levels (66.33 and 43.66 cmH_20 respectively). His ALSFRS-R score dropped to 36 (-2.70%) and 28 (-28%) at the 3- month and 6-month follow up time points respectively. Follow up data out to 12 months will be presented.

Discussion and conclusion: In these two cases we document an immediate effect of EMST on expiratory force generating pressures and global disease status. Specifically, MEPs increased by 41–43% in these patients and ALSFRS-R scores remained unchanged pre vs. post-EMST. Improvements in MEPs did not carry-over beyond three months in the patient who discontinued EMST, however were maintained with continued exercise (Patient 1) out to 8 months.

Strength training of bulbar musculature may be beneficial for improving and maintaining expiratory generating pressures and may impact measures of global disease progression. Further work is warranted to validate these preliminary data in a larger cohort of individuals with ALS.

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P84 NON-INVASIVE VENTILATION INFLUENCES RESTING ENERGY EXPENDITURE IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

MARINOU K[1], BOVIO G[2], CENA H[3], MARIANI P[4], MORA G[1]

[1]*Department of Neurological Rehabilitation,* [2]*Metabolic-Nutritional Unit and Palliative Care Unit,* [4]*Clinical Nutrition Department Salvatore Maugeri Foundation, Pavia, Italy,* [3]*Department of Public Health, Section of Human Nutrition and Dietetics, University of Pavia, Pavia, Italy*

Email address for correspondence: kalliopi.marinou@fsm.it

Keywords: non-invasive ventilation(NIV), energy expenditure, indirect calorimetry

Background: Non-invasive ventilation (NIV) has been demonstrated to be the treatment of choice for respiratory failure in patients with amyotrophic lateral sclerosis (ALS) because it reduces respiratory muscles activity and improves gas exchanges.

Two studies investigated the resting energy expenditure (REE) in ALS patients undergoing invasive ventilation. Both studies found a decreased REE, attributed to the reduced respiratory work load supported by the invasive ventilation. To our knowledge no study compared REE in ALS patients with and without NIV.

Objectives: The primary aim of our study was to determine whether respiratory failure and NIV have an influence on the REE in ALS patients. The secondary aim was to determine whether the Harris-Benedict formula (pREE) correctly predicts the REE in these patients.

Methods: We studied 9 ALS patients (4 M, 5 F, 4 bulbar and 5 spinal onset), mean age 66.1 ± 7.7 years. All patients have been using NIV during sleep for at least 4 months. All patients underwent neurological, respiratory and nutritional evaluation. Nutritional assessment included weight, height, body mass index and bio-impedance analysis and, on alternate days, indirect calorimetry during spontaneous breathing and during NIV, to measure the REE (mREE) in these two conditions.

The mREE was also compared to pREE. Patients were considered hypermetabolic if mREE/pREE was higher than 10%, hypometabolic if the ratio was lower than 10% and normometabolic if the ratio ranged between + 10 % and −10 %.

Results: The mean BMI was 24.54 ± 2.7 kg/m^2 (range 19.2–28.8). The mean mREE obtained without NIV was 1123 ± 181 kcal/day, while mREE obtained during NIV was 1019 ± 180 ($p = 0.036$). The mREE obtained without NIV showed normometabolism in 5 patients and hypometabolism in 4 patients. The mREE during NIV showed hypometabolism in 8 patients, while one patient was normometabolic.

In patients without NIV, pREE overestimated the basal metabolic rate of 14.7 ± 12 %; in patients with NIV, pREE overestimated the basal metabolic rate of 23 ± 8.4 %.

Discussion and conclusion: 1) Respiratory failure produces an increase in mREE probably due to respiratory muscles hyperactivity; 2) mREE is significantly reduced during NIV; 3) pREE overestimates REE in patients both in spontaneous breathing and during NIV; 4) Indirect calorimetry is recommended for nutritional assessment in ALS patients.

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P85 EFFECTS OF EARLY NON INVASIVE VENTILATION ON PULMONARY FUNCTION IN ALS PATIENTS: PRELIMINARY RESULTS OF A RANDOMIZED CONTROL TRIAL

GONZALEZ N[1], PRATS E[1], RODRIGUEZ-GONZALEZ MJ[2], MARTI S[3], LUJAN M[4], POVEDANO MP[1], TURON SJ[1], ANTÓN A[5], CALLE M[6], FARRERO E[1]

[1]*Hospital Universitari de Bellvitge, L'Hospitalet, Barcelona, Spain,* [2]*Hospital Gregorio Marañon, Madrid, Spain,* [3]*Hospital Vall*

Hebron, Barcelona, Spain, [4]*Hospital Parc Tauli, Sabadell, Barcelona, Spain,* [5]*Hospital Sant Pau, Barcelona, Spain,* [6]*Hospital Clinico San Carlos, Madrid, Spain*

Email address for correspondence: efarrero@bellvitgehospital.cat

Keywords: non-invasive ventilation(NIV), early initiation, survival

Background: Non-invasive ventilation has demonstrated an improvement of survival and quality of life in ALS patients. Recent studies (1, 2) suggest that early initiation, in early stages of respiratory muscle weakness, would give a greater increase in survival

Objective: To assess the effects from early use of non-invasive ventilation (NIV) in progression of respiratory muscle weakness, measured by rate of decline in Forced Vital Capacity (FVC).

Methods: A multicentre, randomized, open-label, controlled clinical trial including patients with FVC < 75% who are randomized to: 1; early NIV (treatment initiation after randomization) and 2; standard NIV (treatment initiation when FVC < 50% predicted, presence of orthopnea, and/or PaCO2 > 45 mmHg). Patients were assessed every three months. Variables collected included: anthropometric variables and data of ALS disease, functional respiratory variables (spirometry, arterial blood gases and nocturnal pulseoxymetry).

Non parametric test were used to compare groups at baseline. Differences in the FVC decline between groups, over consecutive visits were compared using U Mann-Whitney and Wilcoxon test was used to analyze differences within each of the two groups.

Results: 41 patients have been included in the trial overall; for the purpose of this study we analysed 25 patients (10 patients in early NIV group and 15 patients in the standard group; 52 % men, mean age 59 (12) years; 92 % of cases the onset of the disease was limb). There were no differences between both groups in anthropometric variables, data of ALS disease, arterial blood gases or nocturnal pulseoxymetry values. During the follow-up we only observed a slight significant decrease in FVC at 6 months in the early NIV group (FVC baseline 69%, FVC 3 months 69%, FVC 6 months 62% p = 0.01) while there was a highly significant and progressive decrease in the standard group (FVC baseline 68%, FVC 3 months 53% p = 0.0001, FVC 6 months 45% p = 0.009). Differences between groups were significant at 3 and 6 months.

Conclusion: Preliminary results in our study show a highly significant beneficial effect of early NIV in ALS patients, slowing-down the progressive decline of Functional Vital Capacity.

Acknowledgements: SOCAP, ISCIII PI11/01209, Esteve-Teijin.

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P86 SAFETY AND TOLERABILITY OF SYSTEMIC BETA-2-ADRENERGIC AGONIST (ALBUTEROL) AS PHARMACOLOGICAL THERAPY IN NON-INVASIVE VENTILATION (NIV)-SUPPORTED AMYOTROPHIC LATERAL SCLEROSIS (ALS) PATIENTS WITH CHRONIC RESPIRATORY FAILURE

BROOKS BR[1,4], KANDINOV B[1], LANGFORD VL[1], LINDBLOM SS[1,4], SANJAK MS[1], WRIGHT KA[1], WARDAL[1,6], HOLSTEN SE[1], FISCHER MP[1], LUCAS NM[1], SMITH NP[1], NICHOLS MS[1], BELCHER SL[1], LARY C[1], NEMETH J[1], RUSSO PC[1], BOCKENEK WL[3,4], BRAVVER EK[1,4], DESAI UG[1,4], STORY SJ[1,4]

[1]*Carolinas Neuromuscular/ALS-MDA Center,* [2]*Department of Internal Medicine - Carolinas Medical Center, Charlotte NC, USA,* [3]*Department of Physical Medicine and Rehabilitation - Carolinas Rehabilitation, Charlotte NC, USA,* [4]*University of North Carolina School of Medicine – Charlotte Campus, Charlotte NC, USA,* [5]*Department of Kinesiology, University of North Carolina - Charlotte, Charlotte NC, USA,* [6]*Cabbarus College of Health Sciences - Occupational Therapy, Concord NC, USA*

Email address for correspondence: benjamin.brooks@carolinashealthcare.org

Keywords: beta-adrenergic agonist, albuterol, non-invasive ventilation (NIV)

Background: NIV decreases the rate of respiratory function decline in ALS patients (1, 2). Few clinical trials have addressed improving respiratory function or extending time before NIV daytime-dependency in these patients (3). Beta-2-adrenergic agonists (albuterol/clenbuterol) have increased vital capacity and neck strength short-term in ALS and spinal muscular atrophy patients (4, 5, 6).

Objective: Clinical audit of use of systemic albuterol in NIV-supported ALS patients at an ALS Multidisciplinary Clinic.

Methods: In this study we carried out a retrospective audit of albuterol utilization in NIV-supported ALS patients from 2010–2013. Vital signs (pulse rate, respiratory rate, blood pressure, vital capacity (% predicted-Knudsen (adult), ALSFRS-R individual or combined bulbar-and respiratory-subscores) were reviewed in 108 NIV-supported ALS patients (55M; 52F) at the start of NIV or at the start of albuterol on NIV and then at 3 and 6 months subsequently. In NIV-supported ALS patients with difficulty maintaining breathing capacity associated with bulbar and respiratory dysfunction, Albuterol was started at a median systemic dose (tablet/liquid) of 6 + 3(SD) mg/day.

Results: Albuterol was tolerated for 6 months in 49/52 (31M; 18F) NIV-supported ALS patients but not in 3 patients (1M, 2F; 94.2% tolerability) with no further VC reduction for 3 months. More male patients (p = 0.0262) with VC below 70% predicted (VC = 61.0% predicted (95%CI = 53.9–68.2)) were given albuterol compared with NIV-supported ALS patients (23M; 32F) not receiving albuterol (VC = 77.3% (95%CI = 70.7–83.9)). ALSFRS-R respiratory, but not bulbar-sub-score was significantly (p = 0.0006) decreased in albuterol treated patients (6.4(95%CI = 5.4–7.3)) compared with patients not receiving albuterol (9.2(95%CI = 8.0–10.6)). Pulse rate increased 10% from baseline but the population variability of this change within the normal pulse rate range was not significant. VC remained unchanged at 3 months (VC = 57.7% (95%CI = 49.8–65.5)) but declined at 6 months on albuterol (VC = 50.0% (95% CI = 35.8–58.5)).

Discussion and conclusion: Systemic albuterol is 94.2% tolerable when used in NIV-supported ALS patients. Male NIV-supported ALS patients more commonly required albuterol. VC was maintained at 3 months without change but not at 6 months in albuterol-treated NIV-supported ALS patients. Further studies are required on pharmacologically enhancing the treatment of NIV-supported ALS patients.

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P87 TIMING OF THE INTRODUCTION OF NONINVASIVE VENTILATION AND MECHANICALLY ASSISTED COUGHING IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

KANO O, TAKAZAWA T, HIRAYAMA T, YANAGIHASHI M, ISHIKAWA Y, MIURA K, HANASHIRO S, SAWADA M, KAWABE K, IKEDA K, IWASAKI Y

Toho University School of Medicine, Tokyo, Japan

Email address for correspondence: osamukano@aol.com

Keywords: mechanically assisted coughing (MAC), forced vital capacity (FVC), peak cough expiratory flow (PCEF)

Background: Since most patients with ALS die from respiratory failure, diagnosis and management of respiratory insufficiency is critical. Forced vital capacity (% FVC) and peak cough expiratory flow (PCEF) are the most common respiratory measurement techniques used for the introduction of non-invasive ventilation (NIV) and mechanically assisted coughing (MAC), respectively, in patients with ALS.

Objectives: To study the timing of introduction of NIV and MAC using %FVC and PCEF, respectively, and compare bulbar onset (BO) to spinal onset (SO).

Methods: A total of 40 patients met the revised El Escorial criteria. We selected patients with a %FVC of $< 50\%$ or a PECF of < 270 L/min. The mean age (SD) of the patients was 59.4 ± 15.0 years in the BO ($n = 10$) group and 66.1 ± 8.8 years in the SO ($n = 13$) group. We evaluated the PCEF approximately every 3 months. The PCEF was determined thrice per measurement, and the maximum value was taken as the PCEF. In addition, the body mass index (BMI) and the Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) score were also studied.

Results: A total of 23 patients were enrolled in this study. A decline in the PECF (< 270 L/min) prior to a decline in %FVC ($< 50\%$) was observed in 7 and 10 patients in the BO and SO groups, respectively. However, with regard to the speech item on the ALSFRS-R, BO patients showed more speech impairment than SO patients (2.1 ± 0.9 vs. 3.3 ± 0.7; $P = 0.04$). No significant difference was observed in the BMI between the 2 groups.

Discussion and conclusion: According to the Report of Quality Standards Subcommittee of the American Academy of Neurology, one of the parameters for considering NIV is a %FVC of $< 50\%$ and the parameter for MAC is a PCEF of < 270 L/min. NIV and MAC are important for improving respiratory function and clearing upper airway secretions. Patients in the BO group as well as most patients in the SO group showed a decline in the PCEF (< 270 L/min) prior to a decline in the %FVC ($< 50\%$), although bulbar symptoms on the ALSFRS-R was different between the 2 groups.

The results of this study suggest that MAC may be introduced earlier than NIV in patients with both BO and SO.

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P88 EVALUATION OF THE COUGHASSIST E70 COMPARED TO COUGHASSIST 3000 IN ADULTS WITH AMYOTROPHIC LATERAL SCLEROSIS

JACKSON C[1], LOTZ G[2], JASKO J[2], BOYCE C[2], SCHROTH M[3], JACKSON L[1], KITTRELL P[1]

[1]*University of Texas Health Science Center San Antonio, San Antonio, TX, USA,* [2]*Phillips Respironics, Monroeville, PA, USA,* [3]*Children's Hospital, University of Wisconsin Hospital and Clinics, Madison, WI, USA*

Email address for correspondence: jacksonce@uthscsa.edu

Keywords: mechanical insufflation/exsufflation, airway clearance, Cough Assist

Background: Pulmonary complications are a major cause of morbidity and mortality in patients with compromised airway clearance mechanisms. Coughing is a protective reflex that helps to remove secretions from the airways. The CoughAssist clears secretions by applying a positive pressure to the airway, then rapidly shifting to a negative pressure. This rapid shift in pressure produces a high expiratory flow rate from the airways, simulating a cough. The E70 further assists by loosening secretions through high frequency oscillatory vibrations during inspiration, expiration, or both.

Objectives: The primary objective of this study was to demonstrate E70 improved usability and comfort compared with subjects' current device over 14 days. Secondary outcomes were to evaluate subjects' global impressions of benefit and to gather information about the use of oscillation.

Methods: Subjects prescribed daily Cough Assist use were recruited and used the E70 for 14 days. Subjects/caregivers completed questionnaires capturing E70 usability and impressions of benefit. Usability questions were based on a 1–5 Likert scale: 1 = strongly disagree and 5 = strongly agree. Global impressions were based on a 1–10 Likert scale: 1 is much better, 5 is no change, and 10 is much worse.

Results: Six adult subjects diagnosed with amyotrophic lateral sclerosis (ALS) and one adult diagnosed with spinal muscle atrophy (SMA) ages 23 to 72 years old were recruited. Based on the usability questionnaire data, subjects or caregivers rated the E70 performance equivalent or better than the Cough Assist 3000 for ease of use at home ($p = 0.047$), ease of use outside the home ($p = 0.184$), durability ($p = 0.017$), and overall satisfaction ($p = 0.002$). The E70 proved superior in sputum production ($p = 0.002$), and ease of breathing ($p < 0.001$). Participants preferred the added oscillation and battery features of the E70 that were lacking in the previous device.

The Global Impression Questionnaire showed non-significant trends that tended to favour the E70 with respect to activity limitations ($p = 0.134$), symptoms ($p = 0.121$), emotions ($p = 0.253$) and overall quality of life ($p = 0.341$).

Discussion and conclusion: The Cough Assist E70, with the oscillation feature, was preferred by subjects and/or caregivers over their previous device for secretion management. The E70 performed equivalent to or better than the Cough Assist 3000 with respect to subject usability and comfort over 14 days.

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P89 SLEEP QUALITY AND PATIENT-VENTILATOR ASYNCHRONY DURING NONINVASIVE VENTILATION IN AMYOTROPHIC LATERAL SCLEROSIS

VRIJSEN B[1], BUYSE B[1], BELGE C[1], VAN DAMME P[2], TESTELMANS D[1]

[1]*University Hospitals Leuven, Department of Pulmonology, LUCS, Leuven, Belgium,* [2]*University Hospitals Leuven, Department of Neurology, Leuven, Belgium*

Email address for correspondence: bart.vrijsen@uzleuven.be

Keywords: non-invasive ventilation (NIV), sleep quality, patient-ventilator asynchrony

Background: In amyotrophic lateral sclerosis (ALS) patients treated with noninvasive ventilation (NIV), sleep quality or sleep architecture has not been shown to be improved (1) and a high patient-ventilator asynchrony (PVA) index was present (2). Prospective studies examining sleep quality by polysomnography (PSG) before and after initiation of NIV in ALS are lacking.

Objectives: To analyse sleep quality and PVA at the start of NIV and one month later.

Methods: Full-video PSG, with incorporation of transcutaneous carbon dioxide ($P_{tc}CO_2$) and Trilogy 100 ventilator software, was used to analyze sleep epoch-by-epoch, respiratory events and PVA breath-by-breath in 19 ALS patients (61 ± 9 years, 10 non-bulbar). After diagnostic PSG, NIV was titrated during 3 consecutive nights. Sleep quality was evaluated at discharge and after 1 month. Data are presented as median and interquartile range.

Results: In non-bulbar patients sleep efficiency (SE), arousal-awakening index (AAI) and amount of rapid-eye-movement (REM) sleep were improved at discharge (SE: 70.9% (62.6–81.1), $p < 0.01$; AAI: 18.9/h sleep (14.3–23.9), $p < 0.01$; REM: 21.7% (18.5–27.9), $p < 0.01$) in comparison to the diagnostic PSG (SE: 60.8% (18.6–73.3); AAI: 43.0/h sleep (33.6–70.1); REM: 3.8% (0.0–10.1)) and remained improved after one month (SE: 74.1% (43.4–85.0), $p < 0.01$; AAI: 19.1/h sleep (10.5–30.4), $p < 0.01$; REM: 20.2% (13.0–23.4), $p < 0.01$). Median time of nocturnal $P_{tc}CO_2 > 55$ mmHg decreased from 42.4% (0.1–96.1) at diagnostic night to 0% (0.0–12.6, $p < 0.05$) at discharge and 0% (0.0–14.2, $p = 0.12$) after one month. Bulbar patients only showed improvement in the amount of slow-wave-sleep (discharge: 25.5% (13.3–38.6) vs 12.3 (1.5–21.7), $p < 0.01$; one month: 21.5% (4.0–28.8), $p < 0.05$) without any improvement in $P_{tc}CO2$. Non-bulbar patients showed a PVA index of 31.8 (2.5–167.8)/h sleep at discharge and 17.5 (2.2–55.2)/h sleep at one month. PVA caused an arousal/awakening of 1.9 (0.4–5.9)/h sleep at discharge and 1.1 (0.1–2.3)/h sleep at 1 month. Bulbar patients had a PVA index of 4.3 (2.7–31.3)/h sleep at discharge and 15.4 (3.9–30.7)/h sleep at one month. PVA followed by an arousal/awakening were 0.3 (0.0–1.1)/h sleep at discharge and 0.4 (0.0–1.8) at one month. No differences were found on the amount of PVA during or outside leak occurrence.

Discussion and conclusion: Non-bulbar patients showed improvements in sleep quality, sleep architecture and $P_{tc}CO_2$, while bulbar patients only showed improvement in SWS. PVA are present in bulbar and non-bulbar patients but have no or minor impact on sleep quality/architecture.

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P90 TRACHEOSTOMY AND INVASIVE VENTILATION IN JAPANESE ALS PATIENTS: DECISION-MAKING AND SURVIVAL ANALYSIS

KIMURA F, TAGAMI M, OTA S, TUKAHARA A

Osaka Medical college, Takatsuk-city, Osaka, Japan

Email address for correspondence: in1110@poh.osaka-med.ac.jp

Keywords: NIPPV, long-term care, tracheostomy invasive ventilation (TIV)

Objectives: To evaluate the factors related to the choice of a tracheostomy and invasive ventilation in amyotrophic lateral sclerosis patients and to determine survival time after a tracheostomy at a single institute in Japan between 1990 and 2010.

Methods: Data for survival time until death or tracheostomy were obtained from 160 patients. Fifty-two patients (33%) underwent tracheostomy/ mechanical ventilation.

Results: Tracheostomy and invasive ventilation prolonged median survival time (74 months), as did non-invasive ventilation (48 months) when compared to a non-ventilation-supported control group (32 months; $p < 0.001$ each). The ratio of tracheostomy/mechanical ventilation in patients > 65 years old significantly increased after 1999 (27%) compared to earlier years (10%, $p = 0.002$). Cox proportional modelling confirmed an age of 65 years as advantageous for long-term survival after a tracheostomy. In univariate logis-

tic regression analysis, factors related to the decision to perform a tracheostomy included: an age of 65 years; greater use of non-invasive ventilation; the presence of a spouse; interval and speed from disease onset to diagnosis/tracheostomy; and preservation of motor function. In multivariate logistic regression analysis, age, shorter duration from disease onset until tracheostomy and the presence of a spouse were independently associated with the decision to perform a tracheostomy. Kaplan-Meier plots revealed longer survival times in patients who resided at home after a tracheostomy compared to patients who stayed at a hospital ($p = 0.007$).

Discussion and conclusion: Several reasons may explain the high frequency and increased rate of TIV over past 20 years. First, frequency of TIV appears higher not only in Japan, but also in Asian countries (12.7–21%). Recent reports have described TIV being performed in around 30% of the overall ALS population from Italy Japanese government covers all costs for home non-invasive/invasive mechanical ventilation at any age for both inpatients and outpatients. Regular follow-up in the same institute may influence the rate of TIV and peer counselling by the Japanese ALS Association (JALSA) has contributed to increased adoption of TIV. The implementation of the LTCI as a new and fundamentally reformed social insurance system in 2000 may have facilitated multidisciplinary team care and an associated increase in use of TIV.

Tracheostomy and invasive ventilation are frequently used in Japan. Various factors impact patients' decisions to have these procedures. This study identified factors related to the decision-making process and post-tracheostomy survival.

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P91 AN UPDATE ON OPINIONS AND BEHAVIORS OF JAPANESE AND AMERICAN ALS CAREGIVERS REGARDING TRACHEOSTOMY WITH INVASIVE VENTILATION (TIV)

CHRISTODOULOU G[1], OGINO M[2], GOETZ R[3], MITSUMOTO H[1], RABKIN J[3], THE AMERICAN AND JAPANESE TIV STUDY GROUP[1,2]

[1]*Eleanor and Lou Gehrig MDA/ALS Research Center, Columbia University Medical Center, New York, New York, USA,* [2]*Department of Neurology, Kitasato University School of Medicine, Kanagawa, Japan,* [3]*New York State Psychiatric Institute & Department of Psychiatry, Columbia University, New York, New York, USA*

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

Keywords: caregivers, tracheostomy invasive ventilation (TIV), Japan

Background: Caregivers play an integral role in the lives and care of patients with amyotrophic lateral sclerosis (ALS). Their opinions can be highly influential throughout the disease course. Past studies have shown highly dissimilar rates of TIV among different countries with Japan exceeding the United States substantially. A multi-site survey assessing Japanese and American patients to determine preferences found that contrary to past reports, Japanese patients were more likely to oppose TIV, demonstrating the influence of other factors in the ultimate decision-making (1).

Objectives: To assess caregiver preferences and their determinants concerning TIV in America and Japan.

Methods: 154 American caregivers from five, geographically distributed ALS Clinics and 66 Japanese caregivers from six sites in several cities completed questionnaires regarding TIV.

Results: Caregivers were similar demographically: mean age was around 57, 70–76% were female, 70–72% were spouses/partners, and 41–47% were working full-time. American caregivers were more likely to report excellent health (59%) compared to Japanese caregivers (16%). More American caregivers reported to share the role of head of household and associated decision-making with their spouses (60%) compared to Japanese caregivers (2%). American caregivers reported more emotional support and someone to confide in than Japanese caregivers (93% vs. 71%) but fell short of Japanese caregivers in obtaining assistance in caregiving from others (35% vs. 52%). More Japanese caregivers knew someone utilizing TIV (32%) than American caregivers (6%). When asked whether they were in favour of TIV, 33% of American caregivers were in favour compared to 53% of Japanese caregivers; 44% of American and 37% of Japanese caregivers were undecided; and 22% of American and 10% of Japanese caregivers were opposed to TIV. The most common reason for being in favour of TIV in the American sample was that the patient could maintain quality of life (79%), while Japanese caregivers believed the patient reaching a milestone to be a main contributing factor (51%). All comparisons listed above were significant ($p < 0.05$). Reasons for opposing TIV did not significantly differ between the two samples.

Discussion and conclusion: As predicted, higher rates of TIV in Japan can be attributed to caregiver and neurologist preferences. These findings solidify the importance of caregivers' roles in patient care. This study looked at intentions specifically; therefore, further studies investigating outcome rates must be considered.

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P92 COMPARISON OF COMMUNICATION ABILITY STAGE WITH ADVERSE CLINICAL SIGNS IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS) ON TRACHEOSTOMY INVASIVE VENTILATION (TIV)

NAKAYAMA Y[1], MATSUDA C[1], SHIMIZU T[2], MOCHIZUKI Y[3,4], HAYASHI K[2], NAGAO M[2], KAWATA A[2], OYANAGI K[5], NAKANO I[2]

[1]*Laboratory of Nursing Research for Intractable Disease, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan,* [2]*Departments of Neurology,* [3]*Departments of Pathology, Tokyo*

Metropolitan Neurological Hospital, Tokyo, Japan, [4]*Department of Neurology, Tokyo Metropolitan Kita Medical and Rehabilitation Centre for the Disabled, Tokyo, Japan,* [5]*Department of Brain Disease Research, Shinshu University School of Medicine, Nagano, Japan*

Email address for correspondence: nakayama-yk@igakuken.or.jp

Keywords: tracheostomy invasive ventilation (TIV), impaired communication, adverse clinical signs

Background: Patients with amyotrophic lateral sclerosis (ALS) on tracheostomy invasive ventilation (TIV) have adverse clinical signs and develop communication impairment with disease progression (1). We proposed a classification of advanced ALS focusing on the patient's communication ability (2) and the classification scheme's utility for nursing and for studies. The classification includes four stages: Stage I = communicates in sentences; Stage II = communicates with one word responses; Stage III = communicates with nonverbal yes/no responses; Stage IV = cannot communicate, except for unreliable yes/no; Stage V = cannot communicate by any means (2).

Objective: To clarify the relationship between the stages of communication ability and adverse clinical signs in ALS patients on TIV.

Methods: We enrolled 46 patients with ALS on TIV at home from 2005 to 2013. We classified them by stage of communication ability at the end point. Data was collected on sex, age of onset, duration of disease, duration of TIV, and time from onset to start of TIV. Retrospectively, we assessed the presence or absence of 16 adverse clinical signs as follows: fatigability of eye movement; dry eye; drooling or dry mouth; megaloglossia; unstable blood pressure; disturbance of thermoregulation; dysuria; nausea; unstable blood glucose; otitis media, pneumonia; urinary tract infections; cholelithiasis; tracheal granuloma; urinary stones; decubitus. Data analysis was performed using SPSS, version 21. The Kruskal-Wallis test was used to compare three groups (stages I, II-IV, and V), and p-values < 0.05 were considered significant.

Results: The patients were divided into three groups: stages I ($n = 21$); II-IV ($n = 19$); V ($n = 6$). Statistical significance between-group differences were noted for the time from onset to start of TIV (I: 59.0 months; II-IV: 27.0 months; V: 24.0 months, $p = 0.039$) and the number of adverse signs per person (I: 4.0; II-IV: 7.0; V: 9.5, $p = 0.0001$). No significance between-group difference was noted for either the disease duration or the TIV duration.

Discussion and conclusion: This is the first report evaluating the multiple adverse signs in ALS patients on TIV. We found that the patients with ALS in advanced stages showed an early requirement for TIV and had more adverse signs. Clinicians should give careful attention to the adverse signs of ALS patients on TIV to prevent communication impairment.

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THEME 5 MULTIDISCIPLINARY CARE AND QUALITY OF LIFE

P93 DIAPHRAGM PACING REFERRAL PATHWAY AND OUTCOMES IN AN ACADEMIC MULTIDISCIPLINARY ALS CLINIC

RAHEJA D[1], WALSH S[2], STEPHENS H[1], 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: diaphragm pacing, respiratory management, screening protocol

Background: Diaphragm pacing (DP) is FDA approved for the treatment of hypoventilation in ALS. Our center received humanitarian use device (HUD) Institutional Review Board (IRB) approval for the implementation of DP devices in ALS. We developed a DP screen based on the recommended guidelines for DP use in ALS. If the patient met the criteria, DP was discussed and written information was provided. Criteria to initiate a discussion of DP as a treatment option included the following: negative inspiratory force (NIF) $<$ -60 cm of H_2O, forced vital capacity (FVC) $>45\%$ of predicted (less if bulbar symptoms present), and goals of treatment (as outlined in our Communication and Treatment Preference Assessment (CTPA) indicating "life extension at all cost" or "life extension with selected treatments". Patients indicating a goal for treatment as "comfort only" and cognitive dysfunction were excluded. If the patient met the screening criteria and indicated an interest in DP, further tests of diaphragm function and respiratory status were undertaken: 1) sniff fluoroscopy of the chest or phrenic nerve stimulation to assess diaphragm movement; 2) arterial blood gas measurement. If the X-ray was normal or showed unilateral dysfunction, or if the blood gas showed hypercarbia ($pCO_2 > 45$), patients were referred to the surgeon for further discussion and evaluation.

Objectives: Examine the referral process and outcomes for DP in a multidisciplinary ALS Clinic.

Methods: All patients meeting definite, probable, probable laboratory supported or possible ALS between April-May 2014 were screened for DP. Evaluation sheets were designed to map the DP referral process of patients seen in the clinic. Descriptive statistics were utilized to examine the referral pathway for DP.

Results: 62 patients were evaluated for DP in the ALS clinic. Of those evaluated, 16 (25%) met criteria for DP to be offered as a treatment option and were referred for further testing. 5 (8%) were eventually referred to the surgeon for discussion of DP placement. Reasons for non-referral for DP included patients wishing comfort, not extension of life (39%), abnormal cognitive screen (20%), not meeting FVC criteria (24%) or NIF criteria (33%).

Discussion and conclusion: Utilizing the recommended guidelines for screening for DP and in addition the use of a CTPA has been a useful method for which to screen for and discuss DP as a treatment option for respiratory support for patients in the ALS clinic. Using both criteria has allowed the clinic team to present treatment options to patients that are in line with their goals for care in ALS.

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P94 ASSESSMENT OF BULBAR FUNCTION IN ALS

SMITH RA[1], PATTEE G[2], PIORO E[3], SCHOENFELD D[4]

[1]*Center for Neurologic Study, La Jolla, CA, USA,* [2]*Neurology Associates, Lincoln, Nebraska, USA,* [3]*Cleveland Clinic, Cleveland, Ohio, USA,* [4]*Massachusetts General Hospital, Boston, Massachusettes, USA*

Email address for correspondence: cnsonline@ymail.com

Keywords: bulbar function, clinical trials, self-report assessments

Objective: To determine the most useful methods for assessing bulbar function in ALS.

Background: The clinical assessment of bulbar function in ALS has not attained the standard of practice that is universally employed for the assessment of limb weakness. The ongoing bulbar function treatment trial offered an opportunity to compare self-report assessments of speech, swallowing, and salivation with 'objective' measures such as timed recording of speech and swallowing.

Methods: At screening and baseline visits, subjects were asked to rate bulbar function using two validated self-rating scales and subsequently, an evaluator measured the time in seconds to swallow 30cc of water, a teaspoon of cereal, and to read the "Rainbow Passage".

Results: The three domains of bulbar function - speech, salivation, and swallowing for the self-report CNS-BFS and evaluator administered ALSFRS-R were highly correlated, as were the total scores for each test instrument. Both the self-report speech domains of the CNS-BFS and the evaluator administered ALSFRS-R compare favourably with the direct measurement of the time needed for subjects to read the Rainbow Passage. An even stronger correlation was noted when speech rate was compared with the total CNS-BFS and bulbar ALSFRS-R scores. In this population of subjects, there was little correlation between speech rate and non-bulbar symptoms. Similarly, the swallowing domains of both the CNS-BFS and ALSFRS-R were highly correlated with timed swallowing of liquids but the CNS-BFS was more informative in the instance of timed swallowing of solids. As might be expected, impaired swallowing was associated with increased difficulty handling secretions in this group of patients.

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Discussion and conclusion: The Center for Neurologic Study Bulbar Function Scale (CNS-BFS), a self-report measure of bulbar function, demonstrates concordance with the bulbar domains of the ALSFRS-R that is administered by an evaluator. The speech domains of the CNS-BFS and the ALSFRS-R are highly correlated with timed speech. The swallowing domain of the CNS-BFS is highly correlated with timed swallowing of solids and liquids. The swallowing domain of the ALSFRS-R is highly correlated with timed swallowing of liquids but is poorly correlated with timed swallowing of solids. With a planned enrolment of 110 ALS subjects, it is anticipated that further refinements in the CNS-BFS will result in a self-report scale that will be clinically useful and a robust treatment trial endpoint.

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P95 5% SCOPOLAMINE OINTMENT IS CLASSICAL BUT STILL USEFUL FOR ALS AND OTHER NEUROLOGICAL DISEASES

NAGASHIMA K[1,2], OGINO M[1], TOMINAGA N[1], UCHINO A[1], OGINO Y[1,3], NISHIYAMA K[1]

[1]*Kitasato University, Sagamihara, Kanagawa, Japan,* [2]*Gunma University, Gunma, Japan,* [3]*Hakone Hospital, Odawara, Kanagawa, Japan*

Email address for correspondence: mieko@kitasato-u.ac.jp

Keywords: scopolamine, sialorrhoea, quality of life

Background: Sialorrhea and the aspiration of saliva often become a problem in neuromuscular diseases. The pasting agent (scopoderm TTS®) used in the USA and EU is not approved in many other countries. Furthermore, originally Scopoderm TTS® is for motion sickness, and it is still unclear about the drug optimum dosage for the salivation.

Objectives: To determine the usefulness and side effects of 5% scopolamine ointment for sialorrhea.

Methods: From October, 2008 to March, 2014, we prescribed 5% scopolamine ointment for sialorrhea to 156 patients with neuromuscular diseases in our hospital. We applied it approximately 0.1 g to the posterior auricles in both sides.

For this study, we analyzed 133 cases in whom we evaluated the effectiveness of scopolamine ointment by VAS scale (divided A (0): not effective, B (1~3): moderate effective, C (4~5): obviously effective, D (6~): extremity effective). These cases include 91 ALS; 27 Parkinson's disease-related disease; 7 multiple system atrophy; 5 spinocerebellar degeneration; 4 cerebral palsy; 4 SBMA; 3 muscular disorder; 3 cerebral vascular disorder; 3 cortico-basal degeneration; and 9 other neurodegenerative disease.

Results: The effectiveness in 133 cases was A:not effective 30 cases; B:moderate effective 11 cases; C: obviously effective 46 cases; D: extremity effective 46 cases. In approximately 77% of cases, 5% scopolamine ointment was effective for sialorrhea. The side effects were observed in approximately 12% of patients. These were local rash (7); tensed sputum (4); dry mouth(4) and others(2). Although only one case complained of light headedness, otherwise there was no systemic side effect.

Discussion and conclusion: Our result shows that the scopolamine ointment was useful for improvement of sialorrhea without severe side effects. We reported that in healthy subjects, blood scopolamine concentration was lower in the 5% scopolamine ointment group than the scopoderm TTS® group. One possible explanation is that 5% scopolamine ointment shows it effects through the local site circulation, thus it causes less systemic side effects. In this point of view, 5% scopolamine ointment might have some advantages over a pasting agent (scopoderm TTS®).Our result shows that the scopolamine ointment was an effective, noninvasive, simple, reversible and easy method.

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P96 AFFECTS OF DYSPHAGIA AND GASTROSTOMY FEEDING ON QUALITY OF LIFE FOR PEOPLE WITH MOTOR NEURONE DISEASE

DOYLE L, MCELLIGOTT K, HARDIMAN O

Beaumont Hospital, Dublin, Ireland

Email address for correspondence: lesleydoyle@beaumont.ie

Keywords: gastrostomy, quality of life, dysphagia

Background: Dysphagia and malnutrition are factors which can negatively affect prognosis and quality of life (QOL) for people with MND. This pilot study aimed to investigate to what extent dysphagia and gastrostomy feeding impacts on QOL for people with MND.

Methods: Over an eighteen month period, people with MND who were scheduled for gastrostomy insertion in one acute hospital completed a questionnaire at three stages. Prior to tube insertion (stage 1), participants (n = 14) completed the SWAL-QOL, which is a validated questionnaire regarding the effects of dysphagia on QOL. This was repeated at approximately six weeks (stage 2) and six months (stage 3) following insertion. At stages 2 and 3, participants also completed the RIG-QOL (a questionnaire devised by the researchers to examine changes in wellbeing relating to gastrostomy feeding based on the format of the SWAL-QOL).

Results: Each participant reported an array of swallowing difficulties including choking on food and liquid as well as problems chewing. At stage 1 (N = 14), all participants felt dysphagia had a moderate or significant impact on their QOL. At stage 2 (N = 11), all reported being at least 'somewhat' glad they had the feeding tube inserted while at stage 3 (N = 4) all participants were 'very' glad that they had a gastrostomy. Every respondent reported a better sense of wellbeing following gastrostomy insertion.

Conclusion: In this pilot study, people with MND reported that gastrostomy insertion had a positive impact on their QOL. This finding supports the need for further research in this area across a larger group of participants.

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P97 ALS SYMPTOMS, DISABILITY AND QUALITY OF LIFE : LITERATURE REVIEW AND MODEL GENERATION

YOUNG C[1,2], AYNSLEY G[2]

[1]*Walton Centre NHS Foundation Trust, Liverpool, UK,* [2]*University of Liverpool, Liverpool, UK*

Email address for correspondence: carolyn.young@thewaltoncentre.nhs.uk

Keywords: quality of life, disability, literature review

Background: The limited efficacy of disease modifiers for ALS/MND focuses management on symptom control and maintaining quality of life (QoL). As yet, there is no overview of how different symptoms may influence QoL in ALS/MND and how all the possible factors interplay.

We conducted a systematic literature review to determine how symptoms influence QoL for people with ALS/MND, in preparation for the Trajectories of Outcome in Neurological Conditions (TONiC) study, a British multicentre study of QoL in MND.

Objectives: This study reviews the published evidence on symptoms influencing QoL, identifying potential direct and indirect effects.

Methods: Literature searches were conducted in PubMed, Science Direct, Science Citation Index, EBSCOHOST, Scopus, CINAHL and PsycInfo to identify primary studies published from 1999 to March 2014 inclusive, assessing how disability or symptoms affect QoL in ALS/MND. The symptom list was derived from the American Academy of Neurology ALS Practice Parameters (1). Studies were excluded if their aim was to evaluate the effect of a therapy on QoL or if they provided no data on correlation between symptom and QoL. Direct effects were determined by correlation coefficients, graded according to standard statistical criteria (2). Indirect effects were factors correlating with a direct effect.

Results: 111 potential studies were identified; of these, 82 met exclusion criteria. This left 29 studies describing overall disability severity and 7 symptoms as showing correlations with QoL. In descending order of strength of correlation coefficients, these were overall disability severity, dyspnoea, fatigue, cognitive impairment, depression, emotional lability, anxiety and muscle weakness. Data on every factor for which more than one study was available was conflicting, in that there was at least one study showing no correlation as well as one or more showing some correlation. Factors indirectly contributing were pain (through low mood, anxiety, overall disability severity) and poor sleep (through dyspnoea and fatigue.) There were no studies providing information on correlations between cramps, sialorrhoea, spasticity, dysphagia or weight loss with QoL. A potential multifactorial model of symptoms influencing QoL directly and indirectly has been developed.

Discussion and conclusion: There is preliminary evidence that several physical and psychological symptoms do directly impact QoL for people with ALS/MND. Comparison of studies was challenging due to heterogeneity in study design and outcome measures. Inconsistencies between studies may reflect methodological variations. Some factors appear to have indirect effects by mediating other factors. Further work is needed to systematically identify potential factors and test these in large studies capable of examining direct and indirect effects.

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P98 THE RELATIONSHIP BETWEEN DYSARTHRIA AND QUALITY OF LIFE IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS)

FEROLDI S[1], GINOCCHIO D[1,2], LUNETTA C[2], SANSONE V[2], SCHINDLER A[3]

[1]*Salvatore Maugeri Foundation, IRCCS, Milan, Italy,* [2]*NEuroMuscular Omnicentre, Ospedale Niguarda Cà Granda, Milan, Italy,* [3]*Department of Biomedical and Clinical Sciences "L. Sacco", Milan, Italy*

Email address for correspondence: sarah.feroldi@gmail.com

Keywords: dysarthria, quality of life, QOL-DyS

Background: Few studies have evaluated dysarthria-related quality of life (QoL) in patients with amyotrophic lateral sclerosis (ALS).

Objective: The aims of the study are: 1) to evaluate dysarthria and dysarthria-related QoL in patients with ALS; 2) to analyze its relationships with patients' age, disease duration, motor functions, depression and general QoL.

Methods: 43 patients with ALS have been involved in the study (21 M, 22F; 33 spinal onset, 10 bulbar onset; age 58 ± 11; disease duration 30.2 months $\pm$ 20.1). Inclusion criteria were Mini Mental State Examination (MMSE) $\geq 24/30$ and preserved reading function. Dysarthria severity and characteristics, ALS severity, depression, dysarthria-related and generic QoL were evaluated respectively by means of the Therapy Outcome Measure(1) (TOM) scale, the Robertson Profile(2), the revised Amyotrophic Lateral Sclerosis Functioning Rating Scale (ALSFRS-r), the Beck Depression Inventory(3) (BDI-II), the QoL of the dysarthric speaker(4) (QoL-DyS), Amyotrophic Lateral Sclerosis Assessment Questionnaire(5) (ALSAQ-40) and the Short Form-36 (SF-36) questionnaire(6).

Results: Dysarthria affected 31/43 patients. Dysarthria severity was mild-moderate in 13 patients, severe in 18 patients. Robertson Profile and QoL-DyS scores were significantly more compromised in dysarthric than in non-dysarthric patients ($p < 0.001$). QoL-DyS showed significant correlation with all the subscales of Robertson Profile ($r = 0.812$). A significant correlation was found between QoL-DyS and dysarthria severity measured in all TOM subscales: impairment ($r = 0.797$), disability ($r = 0.705$), handicap ($r = 0.682$).QOL-DyS showed a strong correlation also with ALSAQ-40 items referring to communication and eating ($r = 0.857$and $r = 0.762$); a significant difference between dysarthric and non-dysarthric patients ($p < 0.001$) was found. QoL-DyS showed no correlation with patients' age, disease duration, mental health or depression. SF-36 scores between dysarthric and non-dysarthric patients were similar, with the exception of the physical activity ($p = 0.021$). ALSFRS-r total score (mean $\pm$ SD 27.2 ± 7.8) between dysarthric and non-dysarthric patients showed no difference, while a significant correlation between QoL-Dys and ALSFRS-r bulbar (mean $\pm$ SD 8.6 ± 3.0) ($r = 0.616$) and respiratory subscores (mean $\pm$ SD 9.5 ± 2.3) ($r = 0.421$) was found.

Discussion and conclusion: Dysarthria severity affects QoL in ALS patients according to QoL-DyS scores. QoL-DyS should be used in clinical practice and research in patients with ALS.

Acknowledgements: The authors report no conflicts of interest. This study was only possible thanks to the participating patients and caregivers.

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P99 MIXED CONDUCTIVE-SENSORINEURAL HEARING LOSS IN PATIENTS WITH ADVANCED AMYOTROPHIC LATERAL SCLEROSIS

GINOCCHIO D[1,2], BAROZZI S[1], MAESTRI E[2], CORBO M[2,3], SANSONE V[2], LUNETTA C[2]

[1]*Audiology Unit, Department of Clinical Sciences and Community Health, Università degli Studi di Milano; Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milano, Italy,* [2]*NEuroMuscular Omnicentre (NEMO), Fondazione Serena Onlus, Niguarda Ca' Granda Hospital, Milano, Italy,* [3]*Department of Neurorehabilitation Sciences, Casa Cura Policlinico, Milano, Italy*

Email address for correspondence: daniela.ginocchio@gmail.com

Keywords: hearing loss, hearing aids, communication

Background: Hearing Loss (HL) has not often been documented in individuals with amyotrophic lateral sclerosis (ALS) (1,2,3).

Objective: The aim of the study is to evaluate the characteristics of HL in ALS patients in advanced stages who complained of hearing difficulties, and to investigate the possibility of using a hearing amplification device.

Methods: Five individuals affected by ALS, who complained of hearing difficulties, were involved in the study (4 M, 1 F; median age 54 years; range 52–72 years). The mean disease duration was 49 months (range 23–227 months). All of them were in the advanced stage of the disease with invasive ventilation, anarthria, dysphagia in enteral feeding and tetraplegia. The patients did not present any co-morbidity factors linked with hearing loss. They all underwent an audiological assessment including otoscopic examination, pure-tone audiometry, and acoustic immittance tests. The degree of hearing loss was classified using standard Pure-Tone Average (PTA). All patients were fitted with monaural digital behind-the-ear hearing aids. A 5-item questionnaire, adapted from the Hearing Handicap Inventory for adults (HHIA) (4) (items: 15, 18, 20, 23, 25), was completed by the patients and caregivers before hearing aid application and 3 weeks after.

Results: All of the patients proved to be affected by bilateral mixed hearing loss. The degree of HL was moderate to severe (right ear: mean PTA: 60.6 dB HL, range: 43.3–73.3 dB HL; left ear: mean PTA: 66.3 dB HL; range: 53.3–73.3 dB HL). The tympanograms were all type B, indicating middle ear effusion. The higher scores obtained at HHIA when using amplification (unaided mean score: 18; aided mean score: 6) indicate a reduction of hearing difficulties with the use of amplification devices.

Discussion and conclusion: In individuals with ALS, HL may be an unrecognized condition because it can be masked by other disabling symptoms, and it may contribute to worsen individual quality of life. Our pilot study showed the presence of bilateral, mixed (conductive-sensorineural) HL in patients during the advanced stages of the disease. Moreover, the study emphasized the efficacy of hearing aids in reducing hearing difficulties. Our findings suggest that hearing sensitivity should be studied in ALS patients from the early stages of the disease in order to define hearing loss incidence, interaction with the different clinical phenotypes and timing of onset.

Acknowledgements: The authors report no conflicts of interest. This study was only possible thanks to the participating patients and caregivers.

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P100 AMYOTROPHIC LATERAL SCLEROSIS AND PAIN

IDRISOGLU HA, POLAT N, IDRISOGLU M

Medical faculty, Istanbul, Turkey

Email address for correspondence: haidris@istanbul.edu.tr

Keywords: pain, depression, quality of life

Background: ALS is a fatal neurodegenerative, progressive disease, with muscle weakness, respiratory failure and bulbar symptoms. Fasiculations and cramps are obvious during progression of disease. Secondary symptoms occur during progression. Pain is important in those secondary symptoms and problems. Pathomechanical properties of pain in ALS patients are missing.

Pain occurs in 20–70% of ALS patients during the case of the disease. Pain is very common as the disease progresses. Pain in ALS disease is described as an unpleasant sensory emotion. Pain becomes acute or chronic and pain in ALS is related to immobilisation in the coint and depressive status. The pain is seen in the arms, shoulders and knees. Aetiopathogenesis of this is not well understood.

Results: We have examined 300 patients over 5 years to determine whether pain occurs. We have established pain in 50 of 300 patients. We have seen that 20% of the patients have pain. There were 24 women and 26 men ALS patients with pain. The age interval was 23–82 mean age was 56.When we looked at the pain localization 20% of 50 patients had hip pain, 1% of 50 patients had neck pain, 15% of 50 patients had shoulder pain.

Discussion and conclusion: Our study suggests that pain is frequent in ALS patients and depressive symptoms with pain relate to poorer QoL. Clinicians should pay more attention to both pain and depressive symptoms in ALS patients considering their effect on QoL. İn this report pain in ALS is mentioned and this will be discussed with literature data.

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P101 PAIN ASSESSMENTS VARY IN A MULTINATIONAL SAMPLE

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, quality of life, multinational research

Background: Recent research has demonstrated that pain is a significant symptom in ALS. Such studies are of patients within specific geographic regions or, most broadly, within one country. Perceptions of pain differ between cultures, health care systems vary, and pain management techniques are not uniform between countries. Such factors have not been explored in persons with ALS.

Objectives: To examine perceptions of pain severity and the relationships between pain, quality of life (QOL), disease duration, and function in a multinational convenience sample.

Methods: Three Canadian, 1 Swiss, 1 Israeli, 1 Scottish and 2 Italian ALS Centers contributed data in an Institutional Review Board approved multinational study of QOL. American data was obtained from a previous multi-center study. All patients completed the ALS Specific Quality of Life-Revised (ALSSQOLR) instrument that includes one item that rates pain by asking "how much of a problem pain has been in the last 7 days" using a scale of 0 (no problem) to 10 (tremendous problem). Data analyzed included the ALSSQOLR pain item, ALS Functional Rating Scale-R (ALSFRSR), and demographics. Analysis of variance (ANOVA) was used to assess the effect of country on pain ratings. Post hoc comparisons were made using the Tukey HSD test. Analysis of covariance (ANCOVA) determined the effects of ALSFRSR, disease duration, and negative emotion QOL on pain ratings. We established pain severity categories including: 0 = no problem; 1–3 = mild; 4–6 = moderate; 7–10 = severe. Chi square test examined country differences in distribution of pain severity.

Results: Data was obtained on 396 US, 76 Canadian, 52 Swiss, 56 Israeli, 22 Scottish and 150 Italian subjects. There was a significant effect of country on pain scores, ($F(5, 752) = 23.79$, $p = 0.000$). Mean US pain scores (2.32, SD = 2.77) were significantly lower than those from Israel (4.34, SD = 3.78), Canada (5.39, SD = 3.6), and Switzerland (6.06, SD = 3.78), but did not differ from those in Scotland (3.18, SD = 3.33) or Italy (3.00, SD = 3.33). The negative emotion QOL domain and disease duration were significant co-variates in the model ($p < 0.01$) whereas ALSFRSR was not. Greater emotional distress and longer disease duration relate to higher pain ratings within groups. The distribution of pain severity varied among countries, X^2 ($15, N = 758) = 110.0$, $p < 0.001$.

Discussion and conclusion: Ratings of pain severity by patients with ALS varied between the countries, and were related to the negative emotion domain of the ALSSQOLR (a measure of psychological morbidity) and to duration of disease, but not to physical function. Cultural factors, health care systems, and pain management techniques may contribute to these differences, and should be further explored as a step toward better symptom management in ALS.

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P102 SELF-PERCEIVED EXPERIENCES OF PAIN IN ALS/MND

ÅKERBLOM Y[1], ZETTERBERG L[2], JAKOBSSON LARSSON B[3], ÅSENLÖF P[2]

[1]*Uppsala University Hospital, Uppsala, Sweden,* [2]*Department of neuroscience, physiotherapy, Uppsala University, Uppsala, Sweden,* [3]*Department of neuroscience, neurology, Uppsala University Hospital, Uppsala, Sweden*

Email address for correspondence: ylva.m.akerblom@gmail.com

Keywords: pain, experience, interviews

Background: Although persons with ALS/MND commonly report pain symptoms (1,2), little is known about how pain experiences vary during the course of the disease and between persons.(3,4) This gap of knowledge hinders the possibilities for development of new pain management strategies.

Objectives: To explore variations in patients' experiences of pain in ALS/MND.

Methods: Sixteen participants (male = 11) with various disease durations and a documented experience of pain were individually interviewed. Transcribed data were analyzed according to qualitative content analysis. Researcher triangulation was used to enhance the credibility of analysis (5).

Results: The most striking result was the great variety in patients' experiences. Results were organized into four categories illustrating areas for this variation; description of pain, factors that contributed to the pain, management, impact on daily life. Pain was perceived to have different levels of seriousness; it was sometimes even the first symptom of the disease. Participants described multiple pain locations and seemed to occur more frequently in the morning, the evening, or at night compared to the wakening hours during the day. Drugs had impact on pain, sometimes enough to reach an acceptable level, however some reported unsatisfactory pain relief. Some participants even exclude drugs because of the side effects. Accepting the feeling of pain or repressing it were coping strategies described. Physical activity was another way of successful coping, but sometimes it worked in reverse order. Consequences of pain were immobility, energy drainage, and decreased well-being. Participants experienced that pain reduced their degree of autonomy. For some patients, it seriously affected their well-being by disturbed sleep and negative emotions.

Discussion and conclusion: The participants' experience of pain varied greatly both within and between the patients. The results indicate a need for individually tailored treatment strategies including monitoring of pain and its consequences during the disease progression.

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P103 MORPHINE USAGE IN ALS PATIENTS ON NPPV DOES NOT MAKE LIFE PROGNOSIS WORSE

TOMINAGA N[1], OGINO M[1], KANEKO J[1], NAGASHIMA K[1], NAGAI M[1], UCHINO A[1], URANO Y[1], OGINO Y[1,2], NISHIYAMA K[1]

[1]*Kitasato University, Sagamihara, Kanagawa, Japan,* [2]*Hakone hospital, Odawara, Kanagawa, Japan*

Email address for correspondence: mieko@kitasato-u.ac.jp

Keywords: end of life care, quality of life, morphine usage, prognosis

Background: About 50% of patients with ALS experience respiratory distress in late stage of illness. Thus many guidelines recommend usage of morphine for that situation. Morphine usage is well recognized in United States and Europa, but in other areas it is not popular yet. One possible explanation is there is fear that morphine could make life prognosis shorter. There are few reports that analyses the effects of morphine for the prognosis on ALS patients.

Objectives: To clarify whether morphine usage has effects on prognosis of ALS.

Methods: Two hundred eighty four ALS patients were admitted to our hospital from 2003/04/01 to 2012/04/01. 47 patients who 1) died in our hospital; 2) had a detail medical history available; 3) were on NPPV longer than 22hrs a day; 4) were on tube feeding were enrolled. Patients were divided into morphine administered and non-morphine administered groups. To focus of effects of morphine usage, other factors which might effect for prognosis such as NPPV and tube feeding were equal between two groups. Duration between the day on which patients needed NPPV usage more than 22hrs a day and death was measured.

Results: Thirty patients with morphine use lived 112.1 days in average, 17 patients without morphine use lived 93.5 days. There is no statistical significant differences.

Discussion and conclusion: Morphine use does not shorten life prognosis in ALS patients. Unfortunately some doctors still have prejudice for morphine use in non-cancer patients. We have to let them know that morphine is safe and effective in palliative care of ALS patients.

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P104 PREVALENCE OF BOWEL AND BLADDER SYMPTOMS ATTRIBUTABLE TO ALS

JERANT P, GIBSON SB, HANSEN A, BROMBERG MB, BILTAJI E, DEKA R

University of Utah, Salt Lake City, Ut, USA

Email address for correspondence: patricia.jerant@utah.edu

Keywords: bowel, bladder, survey

Background: Symptoms of bowel and bladder disturbances are common in both the general population and among ALS patients. There is overlap in the range of symptoms representing true disturbances or normal variations. Prevalence values vary depending upon the range of symptoms queried and whether responses are based on formal survey instruments or by self-report.

Objectives: We undertook a survey to determine prevalence rates of bowel and bladder symptoms and whether the symptoms could be attributed to ALS.

Methods: Two surveys were administered at a single clinic visit: a bowel function survey based on Rome III (1), and a bladder function survey based on Primary Overactive Bladder Symptom Questionnaire (POSQ) (2). Both were supplemented with questions concerning the temporal relation to onset of ALS symptoms or diagnosis.

Results: Most patients were in their 6th decade and were male, and the average time from symptom onset to the survey was 2 years.

Amongst bowel symptoms, 19% answered yes to the general question "do you feel you have constipation now", while 57% met Rome III diagnostic criteria for constipation. 62% noted straining during bowel movements as a new symptom since diagnosis, 38% noted hard or lumpy stools, and 19% acknowledged bowel urgency. Bowel movement frequency did not change with the onset of ALS. A variety of treatments were used and polyethylene glycol was the most effective.

Amongst urinary symptoms, 29% answered yes to the general question "do you feel you have urinary problems now". However, to specific questions, 71% were bothered by urgency, 38% were bothered by frequent daytime urination, 48% were bothered by frequent night-time urination, and 33% were bothered by urge incontinence. While virtually all patients were offered treatment, 60% were satisfied with treatment.

Discussion and conclusion: Bowel and bladder symptoms are a common complaint amongst ALS patients, but a formal survey shows a lesser percentage than informal surveys. Among symptoms self-reported by ALS patients, nearly 50% acknowledge bowel issues and nearly 70% bladder issues (3). Symptoms were also under-reported when patients were asked general questions versus asking about specific constipation and urinary symptoms. From our formal survey there appears to be a range of symptoms, treatment options are only partially successful, and providers need to ask specific questions about symptoms to best treat the patient.

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P105 THE PATIENT EXPERIENCE OF SPASTICITY IN MOTOR NEURONE DISEASE

MILINIS K[1,2], YOUNG C[1,2] , ON BEHALF OF THE TONIC STUDY GROUP

[1]*The Walton Centre NHS Foundation Trust, Liverpool, UK,* [2]*University of Liverpool, Liverpool, UK*

Email address for correspondence: miliniskristijonas@gmail.com

Keywords: spasticity, measurement, qualitative

Background: Spasticity is a common impairment in motor neurone disease (MND). Studies in multiple sclerosis and spinal cord injury have found that spasticity affects patients in diverse and complex ways (1, 2). To date no studies have investigated the patient experience of spasticity in MND.

Objective: To explore the lived experience of spasticity in patients with MND.

Methods: Semi-structured interviews were conducted with a random convenience sample of volunteers who attended an MND clinic and who were experiencing any spasticity.

Results: 11 subjects (7 male) aged 40–83 years (median 51) took part, 7 had a limb onset MND, 3 bulbar and 1 respiratory. Median duration of disease was 36 months (range 1–98). The sample was representative of a range of disabilities (Amyotrophic Lateral Sclerosis Functioning Rating Scale range 25–48). Participants described their spasticity using words such as 'muscle stiffness', 'rigidity', 'tightness' 'cramps' and 'spasms'. Three main themes emerged: (i) physical symptoms, (ii) modifying factors and (iii) negative impact.

(i) Symptomatology of spasticity was classed into three domains: (1a) muscle stiffness, (1b) spasms and (1c) sensory consequences (pain and discomfort).
(ii) Factors that worsened or triggered spasticity were sudden movements, writing, stretching, cold, fatigue and exercise. Heat, massage, relaxation and antispasticity medication were found to relieve the symptoms. Bulbar spasms occurred unprovoked or were triggered by yawning, laughing, speaking and eating.
(iii) Negative impact of spasticity varied across the sample. While some participants reported that spasticity was barely noticeable, for others spasticity significantly affected mobility, activities of daily living, social relationships, sleep and mood. Particularly distressing were bulbar spasms, characterized by sudden laryngeal closure and in the most extreme cases causing interference with breathing. In contrast to previous studies on spasticity in other neurological conditions which found that spasticity may have desirable properties, none of the patients with MND reported positive effects of spasticity on mobility or transfers (1,2).

Discussion and conclusion: Spasticity can have far reaching negative effects on people with MND. The findings also suggest that MND-related spasticity can manifest in unique ways, such as bulbar spasms, which have rarely been described in other conditions. Understanding the symptomatology of spasticity and its consequences is an important step forward in developing comprehensive measures of spasticity in MND in the future.

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P106 SELF-ASSESSMENT OF PHYSICAL THERAPY IN ALS

MEYER R[1], MAIER A[1], KETTEMANN D[1], FUNKE A[1], MÜNCH C[1,3], WALTER B[1], GAJEWSKI N[2], KARNAPP C[2], HILDEBRANDT B[3], MEYER T[1,3]

[1]*Charité - University Hospital, Department of Neurology, ALS/MND outpatient department, Berlin, Germany,* [2]*Charité - University Hospital, Department of Neurology, Project team AmbulanzPartner, Berlin, Germany,* [3]*AmbulanzPartner Sozio-Technologie GmbH, Berlin, Germany*

Email address for correspondence: andre.maier@charite.de

Keywords: physical therapy, self-assessment, multidisciplinary care

Background: Although physical therapy (PT) is widely administered in ALS there are but a few systematic investigations on the type, duration and frequency of PT. The subjective experience of PT has remained largely unknown.

Objectives: To evaluate the patients' subjective experience of physical therapy and their likelihood of recommending it.

Methods: 45 ALS patients (64.4 % male; age: 58.6 years; duration of disease: 37.5 months) were included. We employed the Measure Yourself Medical Outcome Profile (MYMOP2) and Net Promoter Score (NPS) for evaluation. With MYMOP2, the patient defined the subjectively most bothersome symptom and a resulting restriction of activity in relation to their self-assessment of general well-being. These are evaluated on a 6-point scale (6 = lowest). NPS is an evaluation instrument elucidating therapy satisfaction by stating the patient's likelihood of recommending their therapy (1–10, 10 = extremely likely). Over a period of 24 weeks, 27 patients were required to fill in an online score every fortnight which was provided on the web portal AmbulanzPartner.de.

Results: The median time spent weekly on PT was 248 minutes (80–615 min). Motor symptoms in the following regions were perceived as being the most bothersome: arms (33 %), legs (60 %) and the trunk (7 %). At the beginning of the observation period, the symptom scored 3.22 (SD 1.08) and activity restriction 3.18 (SD 1.13) points on the MYMOP2-score. Over time, the severity of symptoms and restriction of function increased significantly. Despite functional deterioration patient satisfaction with PT increased in 48% of patients (n = 13). In 22 % (n = 6), satisfaction decreased and in 30 % (n = 8) it remained the same. Median satisfaction increased from 7.7 to 9.0 NPS points. Patients that became increasingly less satisfied had a higher ALS progression rate ($p = 0.05$) and frequency of treatment and a longer overall term of therapy.

Discussion and conclusion: Despite progression of symptoms and loss of motor function, most of the patients described high or increasing satisfaction with PT. High ALS progression rate was a risk factor for low subjective satisfaction. The dissociation of decreasing motor function and increasing subjective satisfaction supports the concept of palliative PT over the course of the disease.

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P107 HOW STORIES COMMUNICATE DAILY LIVING WITH ALS

JEPPESEN J[1,2], RAHBEK J[1], GREDAL O[1], PLOUG HANSEN H[2]

[1]*The National Rehabilitation Centre for Neuromuscular Diseases, Aarhus, Denmark,* [2]*University of Southern Denmark, Odense, Denmark*

Email address for correspondence: joje@rcfm.dk

Keywords: communication, illness experience, patients' perspective

Background: To complement the clinical and therapeutic knowledge about symptoms, prognosis, and social implications of ALS, health research and care need to develop methods that capture and communicate the unique individual impact on daily living with the disease (1, 2, 3).

Objectives: To explore how stories can communicate experiences of daily living with ALS and compensate the progressive loss of ability to speak.

Methods: Twenty-four interviews at home with six people diagnosed with ALS were transformed into narrative journalistic stories (4). A formal readership was selected by the participant among his or her most significant health professionals. Topics of stories were categorized and selected themes analysed and interpreted.

Results: The stories communicated daily living with ALS as a continuous process of creating a new normality of everyday life. The stories also revealed conflicting views between patient and professionals about information about disease and prognosis (5, 6).

Discussion and conclusion: The narrative journalistic story enhances communication about daily living with ALS by offering a mode of sharing experiences that compensate the progressive loss of communicative abilities. The story can sustain meaning in living with ALS and support patients to appreciate a day-to-day life which is not just a waiting for death. By showing that living with ALS is a constant struggle of leading a life as normal as possible, and how important this hard work is for the patient, narrative journalistic storytelling may educate health professionals that a medical prognosis should be complemented by understanding the individual's unique experience of vulnerability.

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P108 PATIENT COMMUNICATION AND TREATMENT PREFERENCES IN AN ALS CLINIC

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

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

Email address for correspondence: hstephens1@psu.edu

Keywords: goals for care, end of life, advance care planning

Background: The Penn State Hershey Communication and Treatment Preference Assessment (CTPA) was developed in an effort to document elements that would guide the ALS health care team in treatment and end of life discussions.

Objectives: Describe communication and treatment preferences of patients in an ALS Clinic as captured by the CTPA.

Methods: Completed CTPAs forms from patients who had given consent to an IRB approved ALS data repository were reviewed for descriptive analysis.

Results: 37 CTPA patient forms were reviewed comprising the sample (65% men, mean ALS Functional Rating Scale-R score = 28.76 (SD = 10.22), average forced vital capacity 61%, and mean ALS-Specific Quality of Life total score of 6.92/10. These statistics match previously reported norms for our ALS clinic. Over 90% of the sample had in place a living will and financial power of attorney (POA) while 75% had in place a healthcare POA and 67% an advance directive. Most patients (81%) preferred to make health care decisions together with family and the medical staff. Few wished to make decisions solely with medical staff (14%) or delegate decision-making to others (5%). Treatment goals of the sample were comfort, not life extension (49%), life extension with selected treatments (40%), and less frequently to extend life at all costs (11%). Half of the sample (50%) preferred information presented in a basic way, while the other half preferred detailed information. 97% of the sample wished to know when their condition has worsened and life expectancy would be short. Most frequent treatments that patients wanted to discuss during the clinic visit included experimental research (50%), feeding tube (50%), respiratory support (43%), diaphragm pacer (36%), and ventilator support (29%). After reviewing the completed forms, advance directives were discussed with 25% of the patients and living wills were discussed with 19%. Financial and Health care POAs were less frequently discussed (< 14%). One third of the patients made a treatment decision during the visit (feeding tube (40%), diaphragm pacer and ventilator support (30%) and experimental research (10%)).

Discussion and conclusion: The CTPA provides structure to the ALS clinic visit and prioritizes discussion of treatments based on patient request and need. The form provides an opportunity to present information in a framework that is most useful to patient's style preferences. The CTPA is a vehicle for assessing the presence of legal documents and providing information to encourage completion of these documents. Most importantly, the CTPA has guided our team to present treatment options that are consistent with the patient's explicit goals for care.

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P109 A QUALITATIVE STUDY TO CONSTRUCT A PREDICTIVE SCALE ON INTERNAL CONFLICT FOR PEOPLE WITH AMYOTROPHIC LATERAL SCLEROSIS

NAKAI M[1], NARITA Y[2]

[1]*Suzuka University of Medical Science, Suzuka-city, Mie prefecture, Japan,* [2]*Mie University, Tsu-City, Mie prefecture, Japan*

Email address for correspondence: mnisi@clin.medic.mie-u.ac.jp

Keywords: internal conflict, predict scale, specialists' perception

Background: People (patients and their families) with amyotrophic lateral sclerosis (ALS) frequently confront difficult decision-makings for symptom management or their quality of life. A number of patients and their family members experienced internal conflict (IC) which was defined as disagreements and opposition on various issues regarding patients care. IC was regarded as one of the factors which have negative impacts on their well-being. To predict IC at early stage will bring some benefit to support both patients and family members.

Objectives: The aim of this study was gathering specialists' opinion to construct a predictive scale on an internal conflict for people with ALS.

Methods: In the first step we generated a draft scale of 54 items to predict IC for ALS patients and their family which was based on a data derived from pre-existing studies. Second step, pre-test and semi-structured interview were conducted by ten specialists (male:5, neurologist = 6, domiciliary nurse = 3, clinical psychotherapist = 1) between July 2013 and October 2013. The audio recorded interviews were transcribed, coded and analyzed according to a qualitative study.

Results: Four points were derived from the interviews. 1) Comments about the problem of inadequacy on item wording and response format, redundancy, compound sentences, which valid the scale contents and lead to more comprehensive scale items. 2) Factors which were recognized as mainly correlated of IC in ALS by health professionals: family communication, decision making style, role changes, values and understanding of the disease. 3) Consideration for impact of interview to patients and family: Three participants mentioned about burden associate with the interviews, 1 of them detected potential risk of burden. Domiciliary nurses suggested that the interview should open up communication within patients, family members and health professionals. 4) Actual condition of IC which experienced by health professionals were detailed.

Discussion and conclusion: Through the pilot test to the specialists and the interviews, we had many suggestions to revise the draft scale and to improve the procedure. All participants had experienced IC in some ALS cases. Factors which were recognized to be associated with IC in ALS from specialist' perspectives were extracted. Interview by the IC scale may be a beneficial support tool to identify IC and trigger to discuss among stakeholders. Due to awareness of IC may have seemed to depend on the background of each specialist, existence of IC should be determined by all stakeholders' perception.

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P110 ANALYSIS OF DEPRESSION AND ANXIETY IN ALS PATIENTS

TEJADO DA, PÉREZ RS, MORENO MJ, TURÓN SJ, POVEDANO PM, PAIPA MA, LAZO LA TORRE C, MELÓN BA

Hospital Bellvitge, Barcelona, Spain

Email address for correspondence: clazolatorre@gmail.com

Keywords: anxiety, depression

Background: Chronic diseases are often accompanied by anxiety and depression. Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that leads the patients to a high grade of functional dependence, consequently a state of depression and anxiety.

Objectives: Analyse the grade of anxiety and depression using the Goldberg scale in patients with ALS disease attended in by ALS Multidisciplinary Unit in a tertiary center of Barcelona.

Methods: Goldberg Scale (Goldberg and al, 1198, Spanish version GZEMPP, 1993) was collected during the period of May 2013 to March 2014 in adult patients with sporadic ALS disease in different stages of disease evolution. It excluded patients with antecedents of psychiatric disease or cognitive involvement.

Results: There were 89 patients, 43 were men and 46 women and 60 patients had a spinal onset and 29 a bulbar onset.

Mixed syndrome is the most frequent in the overall cohort (88.2%). According by sex, the depressive syndrome was more frequent in women (36.9%) and mixed syndromes (AS + DS) predominated in men (53%). According by phenotype, the mixed syndrome is slightly increased in patients with bulbar involvement (45% and 43%) without statistical significance.

In order to homogenize the sample we classified the cohort into two subgroups of age, with cut-off line of 45 years, we then obtained 15 patients younger than 45 years and 74 older. Mixed syndromes predominated in both subgroups without difference in age; however depressive syndrome is clearly more frequent in younger patients (40%).

To correlate the time of evolution of disease, we classified the cohort into three subgroups: patients in process of diagnosis and/or very recent diagnostic (11); with disease duration of less than three years (35) and with more than 3 years of duration of disease (43). The mixed syndrome was more frequent in the first subgroups (72%). Patients with more than 3 years of disease have more frequency depressive syndrome, but in the overall cohort the frequency of all the syndromes tend to decrease over the course of disease.

Discussion and conclusion: Mixed syndrome predominated in all patients, quite more frequent in bulbar patients and clearly more frequent in earlier stages of disease. Depressive syndrome is more frequent in younger patients and in later stage of disease. The overall disorders tend to be less frequent in later stages of disease.

Our results show that people in process of diagnosis are more susceptible to anxiety-depressive disorders, this would be related with a period in which the disease is not yet accepted, and younger patients are more vulnerable. Also the longer disease duration the less prevalent they are, would be correlated with a better acceptation of disease.

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P111 ILLNESS REPRESENTATIONS AND COPING STRATEGIES AS DETERMINANTS OF QUALITY OF LIFE IN AMYOTROPHIC LATERAL SCLEROSIS: FINDINGS FROM A GREEK SAMPLE

FERENTINOS P[1], RENTZOS M[2], CHRISTIDI F[2], ZOUVELOU V[2], ALEXAKIS T[2], ATHANASSOULI P[2], PAPARRIGOPOULOS T[1], EVDOKIMIDIS I[2]

[1]*1st Department of Psychiatry,* [2]*1st Department of Neurology,University of Athens, Eginition Hospital, Athens, Greece*

Email address for correspondence: pferentinos@med.uoa.gr

Keywords: quality of life, illness representations, coping strategies

Background: Leventhal's Common Sense Model (CSM) of illness representations hypothesizes that individuals create on the basis of a priori information cognitive and emotional representations of their illness, which help shape coping strategies and behaviours to manage their problem. Both illness representations and coping strategies are important determinants of disease state, and physical and emotional adjustment. The inter correlations between illness representations, coping strategies and physical and emotional outcomes are understudied in patients with amyotrophic lateral sclerosis (ALS).

Objectives: The present study examines the effect of illness representations and coping strategies on physical and psychological adjustment (as measured by physical and mental components of health-related quality of life) in a sample of Greek patients with ALS.

Methods: We included 56 consecutive patients diagnosed with ALS on the basis of El Escorial revised criteria. Patients with severe respiratory insufficiency or dementia were excluded due to difficulty in going through psychometric evaluations. Demographic characteristics and disease-related parameters (disease duration, time since diagnosis, body area affected, bulbar onset, ALSFRS scores) were recorded. Patients' illness representations were extracted with the revised Illness Perception Questionnaire (IPQ-R), which consists of identity, timeline (acute/chronic and cyclical), consequences, personal control, treatment control, illness coherence and emotional representations dimensions. Coping strategies were assessed using the revised Ways of Coping Checklist (WOC), which consists of five broad factors: positive approach (positive reappraisal and problem-solving efforts), seeking social support, wishful thinking, avoidance/distancing, and confrontive coping. Quality of life (QoL) was evaluated using the SF-36 Health Survey (SF-36), measuring eight generic concepts: physical functioning, physical role, bodily pain, general health perceptions, vitality, social functioning, emotional role and mental health. All measures have been previously validated in the Greek population. A two-step clustering method was employed to classify ALS patients into clusters based on SF-36 scores. A hierarchical logistic regression approach was then used to identify predictors of SF-36 cluster membership belonging to each of four domains: demographic characteristics, disease-related parameters, IPQ-R dimensions and WOC factors.

Results: Clusters 1 and 2 were obtained, with 32 and 24 patients, respectively. Cluster 1 patients scored significantly lower on all SF-36 subscales. In the final logistic regression model, education (years), legs affected, consequences IPQ-R dimension, problem-solving efforts and wishful thinking WOC strategies were tested as predictors of SF-36 cluster membership. Legs affected and consequences IPQ-R dimension were significantly associated with lower QoL scores ($p = 0.002$ and 0.013, respectively) while problem-solving efforts were associated with higher QoL ($p = 0.014$); Nagelkerke $R^2 = 0.695$.

Discussion and conclusion: In a Greek sample of patients with ALS, lower limb involvement and a negative perception of the impact of the disease on overall QoL and functionality were associated with worse physical and psychological adjustment; a problem-focused coping approach was linked to better QoL.

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P112 COGNITIVE CHANGE AND QUALITY OF LIFE IN AMYOTROPHIC LATERAL SCLEROSIS

NIVEN E[1,2], NEWTON J[1,3], BAK T[1,3], STEPHENS H[4], SIMMONS Z[4], ABRAHAMS S[1,3]

[1]*The University of Edinburgh, Edinburgh, UK,* [2]*Euan MacDonald Centre for Motor Neurone Disease Research, Edinburgh, UK,* [3]*Anne Rowling Regenerate Neurology Clinic, Edinburgh, UK,* [4]*Penn State Hershey Medical Center, Hershey, PA, USA*

Email address for correspondence: E.H.Niven@ed.ac.uk

Keywords: cognitive, assessment, quality of life

Background: Cognitive change is known to occur in up to 50% of patients with ALS, affecting abilities including thinking, language and/or behaviour. However, the relationship between cognitive change and patients' self-reported quality of life (QOL) has not been a focus of research.

Objectives: To investigate the relationship between cognitive profile and self-reported quality of life (QOL) in patients with ALS.

Methods: 30 non-demented patients with ALS (age 64.50 + 9.23 years) completed both The Edinburgh Cognitive and Behaviour ALS Screen (ECAS)(1) - developed for ALS patients with physical disability - and the ALS Specific Quality of Life-Revised instrument (ALSSQOL-R)(2). The ECAS's multi-domain screen was designed to detect the specific profile of cognition and behaviour changes characteristically found in ALS, providing ALS-Specific (executive functions, fluency, and language), ALS Non-specific (memory and visuospatial) and total scores. The ALSSQOL-R is designed to reach beyond physically dominated perspectives of QOL and provides a single item score and an average total ALSSQOL-R score as well as six domain scores (negative emotion, interaction with people and the environment, intimacy, religiosity, physical symptoms, and bulbar function) representing answers to 50 wide-ranging and disease-specific items.

Results: Data showed that patients who presented as impaired via ECAS screening (displaying impairment on ALS-Specific or ECAS total scores) did not provide significantly lower QOL single item scores ($M = 6.27$, $SE = 0.75$) than those who showed no impairment ($M = 6.42$, $SE = 0.45$; $t(28) = -0.82$, $p > 0.05$). Nor did patients with impairment ($M = 6.33$, $SE = 0.44$) provide average total ALSSQOL-R scores - derived from responses to all answered questions - than patients not presenting with impairment ($M = 6.34$, $SE = 0.32$; $t(28) = -0.03$, $p > 0.05$). However, the inclusion of subdomain scores allows a more detailed representation of QOL assessment in these two patient sub-groups.

Discussion and conclusion: According to our results, patients with ALS who present with impaired performance at

cognitive screening may provide similar overall and similar averaged totals of self-reported QOL assessment. However, it is important to give consideration both to patients' cognitive status and to the nuanced, disease-specific subscale information available through the ALSSQOL-R instrument. The use of these two short screens together provides a means to allow a fuller understanding of the obtained profiles.

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P113 WHAT IS UNIQUE ABOUT QUALITY OF LIFE IN MOTOR NEURONE DISEASE?: A QUALITATIVE QUERY

YOUNG C[1,2], ANDO H[3,1], COUSINS R[3]

[1]*Walton Centre NHS Foundation Trust, Liverpool, UK,* [2]*University of Liverpool, Liverpool, UK,* [3]*Hope University, Liverpool, UK*

Email address for correspondence: carolyn.young@thewaltoncentre.nhs.uk

Keywords: quality of life, end of life, qualitative study

Background: Quality of Life (QoL) is an important parameter in neurological clinical care. The debate about generic and disease specific measures of quality of life reflects uncertainty about whether there are unique aspects to understanding QoL in different diseases. If there are congruities to different disabling neurological diseases, it may be credible to extrapolate from more common and better-studied conditions, like multiple sclerosis (MS) to rarer diseases like motor neurone disease (MND). In contrast, divergence across neurological conditions would suggest that strategies to maximize QoL must be disease specific.

Objectives: In this analysis our aim was to explore psychosocial factors affecting QoL in MND in comparison with MS.

Methods: The study employed both semi-structured interviews and focus groups. Each focus group consisted of either MND or MS. All interviews and group discussions were audio recorded and transcribed verbatim for thematic analysis. Themes were inductively developed within a condition before they were compared across the two conditions for cross-sectional exploration.

Results: Data was collected from 40 people with MND: 26 interviews (male = 14; mean age = 64yrs; mean illness duration = 1.6yrs); 14 in focus groups (male = 9; mean age = 62yrs; mean illness duration = 3.5yrs); and from 61 people with MS: 43 interviews (male = 16; mean age = 52yrs; mean illness duration = 15yrs); 18 in focus groups (male = 6; mean age = 47yrs; mean illness duration = 9yrs). A codebook for each condition was developed for the analysis and saturation was achieved in both MND and MS.

Whilst the analysis identified the same psychosocial domains to be important for QoL irrespective of disease, there was an aspect unique to MND of it being a terminal condition. Both MS and MND patients valued their lives, but MND patients recognized their shorter life expectancy and differed from MS patients because thoughts of imminent death adversely impacted QoL. Participants' concerns about death were not restricted to their own death, but extended to its impact on significant others. The differences between MND and MS were further observed with regard to factors that were beneficial to QoL. Although the influence of environmental factors and spirituality were found in both conditions, additional implications of these factors were found amongst the MND group: it was found that the importance of environment included the place of death, and spirituality was described to provide hope even beyond death.

Discussion and conclusion: The current study reveals complexity in ascertaining QoL across neurological diseases. Despite distinct illness trajectories, the same psychosocial factors were identified to be important for QoL in MND and MS. Nevertheless, MND as a terminal condition was found to further challenge patient's QoL. The findings confirm the importance of addressing this aspect of the condition in addition to commonly investigated psychosocial factors.

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P114 THE DEVELOPMENT OF A CONSENSUS PAPER ON PALLIATIVE CARE IN NEUROLOGY – THE IMPLICATIONS FOR ALS CARE

OLIVER D[1], BORASIO GD[2], VOLTZ R[3], CARACENI A[4], DE VISSER M[5], LORENZL S[6], VERONESE S[7], GRISOLD W[8]

[1]*University of Kent, Chatham, UK,* [2]*Center Hospitalier Universitaire Vaudois, Lausanne, Switzerland,* [3]*University Hospital, Cologne, Germany,* [4]*Fonazione IRCCS, Milan, Italy,* [5]*AMC, Amsterdam, The Netherlands,* [6]*University Hospital, Munich, Germany,* [7]*FARO, Turin, Italy,* [8]*Kaiser Franz Josef Hospital, Vienna, Austria*

Email address for correspondence: drdjoliver@gmail.com

Keywords: guidelines, care planning, training

Background: A joint working group of the European Association for Palliative Care and the European Federation of Neurological Societies has produced a Consensus paper on the available evidence for the palliative care in progressive neurological disease. This has been a generic paper looking at all progressive neurological disease – including ALS/MND, multiple sclerosis, Parkinson's disease, stroke and primary brain tumours. The principles need to be considered separately for all disease groups as well – including ALS/ MND.

Objectives: To show the important areas for consideration in the palliative care for people with ALS/MND.

Methods: A literature search was undertaken including the main electronic databases and looking at the main areas of palliative care and neurology. Two investigators then looked at this literature, determined seven main areas and developed a draft list of papers. These were then commented on by a small group and then more widely until a consensus was developed.

Results: The seven main areas all apply to the care of people with ALS/MND, and it could be argued that with the short

prognosis of ALS/MND and the variable rate of progression this approach should be from the time of diagnosis.

The areas of recommendation are: Palliative care should be considered early in the disease trajectory; the assessment and care should be provided by a multidisciplinary team approach, with access to specialist palliative care; communication should be open with patients and families and advance care planning is recommended. This should be as soon as possible in view of the likelihood of difficulties in communication and the development of cognitive change in ALS/MND; symptoms – physical and psychosocial – should be managed actively and appropriately; care needs should be assessed and carers supported before and after death. Professional carers should receive education, support and supervision to reduce the risks of emotional exhaustion; there should be repeated and continued discussion about end of life issues and discussion of patients' wishes and aims. The recognition of the deterioration and dying phase will allow appropriate management and intervention; palliative care principles should be included with the training and continuing medical education of neurologists and palliative care professionals should understand the issues for neurological patients.

Discussion and conclusion: The recommendations within the Consensus document can be seen to apply to the care of people with ALS/MND and there is a challenge to ensure that these principles are extended as widely as possible to support people with ALS/MND and their carers.

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P115 END OF LIFE IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS): A REVIEW

CONNOLLY S[1], GALVIN M[2], HARDIMAN O[1,3]

[1]*Trinity College Dublin, Dublin, Ireland,* [2]*Dublin City University, Dublin, Ireland,* [3]*Beaumont Hospital, Dublin, Ireland*

Email address for correspondence: sheelahconnolly@gmail.com

Keywords: end of life, dying, decision-making

Background: ALS is an incurable condition with approximately 70% of those affected dying within three years from symptom onset. However, there has been relatively little discussion about the end-of-life phase; perhaps because the thrust of medical education is generally towards curative therapies and end-of-life discussions require a different perspective, including coming to terms with the limitations of medical interventions as life promoting initiatives.

Objectives: The aim of this review is to identify and review the most important themes in the context of management of end of life in ALS.

Methods: Relevant end-of-life themes were identified by a literature search and through discussion with health professionals in Neurology and Palliative Care in the Irish National Centre for ALS. Relevant publications were identified through a search of Medline and Pubmed using the following Keywords: motor neuron disease (MND), amyotrophic lateral sclerosis (ALS), end of life, dying, death, decision-making, advance care directive and euthanasia.

Results: Through discussion with health professionals and literature search five major themes were identified - (1) importance of end-of-life discussions and decisions; (2) use of life prolonging interventions and technologies; (3) life limiting interventions (4) the experience of dying and (5) best practice at end of life.

Discussion and conclusion: Early and honest discussion of end-of-life issues allows time for reflection and planning, can obviate the introduction of unwanted interventions or procedures, and help alleviate fear around dying. Advance care directives can provide patients with options to exercize autonomy regarding preferred end-of-life management strategies, although their legal validity and use varies from country to country. Preferences around end-of-life interventions and use of technologies vary, and it is important that health care professionals respect patients autonomy. Formal care at the end of life should aim to maximize quality of life of both the patient and caregiver and where possible incorporate appropriate palliation of distressing physical, psychosocial and existential distress. Training of health care professionals should include the development of skills that help to sensitively manage the inevitability of death in terminal illnesses such as ALS.

While the importance of end-of-life care is increasingly being recognized, the particular needs of ALS patients and their families require more attention. Successful management of patients through the end-of-life period can be challenging for the healthcare professional, trained in promoting life and preventing death.

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P116 ETHICAL ASPECTS TO CONSIDER IN COUNSELLING FOR END-OF-LIFE DECISION MAKING

LULÉ D, BADURA-LOTTER G, BÖHM S, LUDOLPH AC, FANGERAU H

University of Ulm, Ulm, Germany

Email address for correspondence: dorothee.lule@uni-ulm.de

Keywords: end of life, decision-making, palliative care, ethics

Background: Optimized patient care is the primary goal of modern medicine. However, the definition of the optimum is often difficult. ALS is a challenging diagnosis with many different aspects to consider in clinical counselling: decisions in the course of the disease regarding acceptance or denial of life prolonging (invasive and non-invasive ventilation, percutaneous endoscopic gastrostomy) and life shortening treatments (Euthanasia and physician assisted suicide) have to be considered. Cultural and ethical factors might have a strong impact on palliative care decisions and might explain the variety of decisions between countries. In our project, we analyse decision making processes in ALS with special focus on cultural and ethical factors (eg, the dominance of different ethical principles like beneficence, autonomy, non-maleficence and justice).

Methods: Based on two different longitudinal studies on decision making in ALS, an ethical analysis of decision factors was performed. In a first approach, n = 93 ALS patients were interviewed three times in the course of one year and in a second approach n = 100 ALS-patients were interviewed twice in the course of one year with questionnaires concerning their decisions for life prolonging or life shortening treatments. Possible cultural and ethical determinants were acquired such as subjective feeling of being a burden, subjective feeling of autonomy and family bonding. Furthermore, determinants such as subjective quality of life, depression

strategies in coping, religiousness and varies factors of cognition were measured.

Results: Key ethical factors of decision making were feeling of being a burden, family ties and personal thrive for autonomy. Furthermore, many patients were initially unable to decide with regards to life-prolonging treatments, as they were unable to anticipate the therapeutic and clinical relevance of the decision. Furthermore, cultural aspects such as religion have to be considered in clinical counselling. It could be shown that autonomy plays at least a double role in counselling and medical treatment: respect for autonomy as a core ethical principle for health care providers, and autonomy as independence, where it can be seen as a coping strategy and therefore belonging to their quality of life.

Discussion and conclusion: Ethical principles have to be considered in clinical counselling: beneficence (timely provision of appropriate therapeutic treatments to sustain good quality of life), autonomy (patients try to harmonize antagonistic wishes of their need for autonomy and their need for participation of family members in decisions), non-maleficence (no life prolongation if it is considered as suffocation) and justice (every patient has the same rights to prolong or shorten life). Dynamic counselling to balance therapeutic relevance, patients' personal needs (autonomy and family bonding) and cultural determinants (eg, religion) should be the primary goal of modern medicine to match actual needs of patients.

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P117 ASSISTIVE DEVICES IN ALS – ANALYSIS OF 3 YEARS OF MANAGED CARE

FUNKE A[1], MÜNCH C[1,2], WALTER B[1], GAJEWSKI N[3], KARNAPP C[3], HILDEBRANDT B[3], KETTEMANN D[1], MEYER R[1], MAIER A[1], MEYER T[1,2]

[1]*Charité – Universitätsmedizin Berlin, Neurologische Hochschulambulanz, Ambulanz für ALS und andere Motoneuronenerkrankungen (AAM), Berlin, Germany,* [2]*Charité – Universitätsmedizin Berlin, Neurologische Hochschulambulanz, Projektgruppe AmbulanzPartner, Berlin, Germany,* [3]*AmbulanzPartner SozioTechnologie GmbH, Berlin, Germany*

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

Keywords: assistive device, managed care, health politics

Background: ALS is a disease prototypical for the medical need of assistive devices such as mobility and transfer aids or advanced communication systems. Despite the high demand and widespread use, there are few systematic investigations into of assistive devices in ALS.

Methods: Since 4–2011, the provision of assistive devices has been coordinated following a managed care concept which comprises case management and an internet-based management portal ("outpatient partner"; OPP). The digitalisation of care processes allows for the electronic capturing of "real life" data for the provision of assistive devices in ALS. Subject of analysis were the type and use of devices typically applied in ALS and the rejection rate by the health insurance companies in Germany.

Results: Between 3–2011 and 3–2014, 1,254 ALS patients at the Charity were coordinated on the internet platform OPP. In the process, 7,394 assistive devices were prescribed. The overall rejection rate by health insurance companies was 22.3%. The distribution based on the number of assistive devices (AD) per patient was as follows: 1–3 AD 46.9%; 4–10 AD 39.3% as well as > 10 AD 13.8%. The frequency distribution of medical indication (prescription rate) and rejection rate for ALS typical AD was the following: orthotics n = 863 (9% rejected); advanced electronic communication aids n = 561 (26% rejected); mobility exercise equipment n = 104 (43% rejected); multifunction wheelchairs n = 203 (40% rejected), electric-powered wheelchairs n = 489 (33% rejected). As for the rejection rate for electric-powered wheelchairs there were distinct regional differences; ie, Berlin (39%), and the federal states of Brandenburg (28%), Mecklenburg-Western Pomerania (40%), Saxony-Anhalt (32%) and Saxony (34%), respectively.

Discussion and conclusion: The predominant assistive devices in ALS are orthotics, communication aids and electric-powered wheelchairs. The acceptance rate of AD prescribed by ALS neurologists is highly variable among health insurance companies (rejection rate of 9 to 43%). The relative risk of rejection for an electric-powered wheelchair for patients in Berlin was 1.41 as compared to patients from the neighbouring state Brandenburg. High rejection rates for individual groups of assistive devices as well as regional differences in their provision point to a pronounced need for research with high relevance to health policies. There is also further need for research into managed care with regard to the utilisation rate and satisfaction relating to the provision of assistive devices.

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P118 EQUIPMENT UTILIZATION IN THE ALS/MND POPULATION – TRENDS AND TIMING

ALLRED P[1], FELDMAN S[2]

[1]*Cedars Sinai Medical Center, Los Angeles, California, USA,* [2]*Drexel University College of Medicine, Philadelphia, Pennsylvania, USA*

Email address for correspondence: peggy.allred@cshs.org

Keywords: equipment utilization, quality of life, proactive care

Background: Equipment use by those with ALS/MND has long been established as a mechanism for enhancing safe function and improving quality of life. Just as ALS/MND has highly variant presentations and progression rates, the types and timing of equipment utilized by those with ALS/MND also varies. Multidisciplinary teams and coordination of care have greatly enhanced proactive equipment recommendation, but consistency in the timing of recommendations based on ALS/MND disease progression parameters has not been established. Equipment for those with ALS/MND in the US must often be deemed medically necessary before being paid for by insurance companies. Establishing trends and timelines for equipment utilization based on functional measures may assist in procuring equipment earlier. These timelines may also assist patients, families, and outside care teams to increase awareness of equipment needs over the disease

Objective: The objective of our work is to investigate what type of equipment is being utilized by individuals with ALS/MND and at what time point in the disease duration to potentially develop predictive algorithms for equipment recommendations.

Methods: Retrospective chart review was performed at two large ALS centers in the US. Information on the types of equipment recommended, prescribed, and/or utilized by ALS/MND patients throughout their disease duration was collected. Disease parameters such as ALS Functional Rating Scale –Revised scores, vital capacity measures, strength, and disease duration were correlated with the time of equipment recommendation and use.

Results: Proactive recommendation of equipment was evident in the documentation, but notes regarding the utilization of recommended equipment were highly variable. Specific timelines for equipment recommendation and correlations with disease progression parameters will be provided in the presentation.

Discussion and conclusion: Proactive management of symptom progression is a primary goal in ALS care. Establishing timeframes for equipment utilization based on commonly collected disease parameters may allow practitioners to prepare people with ALS/MND for equipment use prior to emergent need. Standardized flow sheets of equipment recommendations and actual utilization are suggested as a mechanism for consistent and accurate documentation across all medical records.

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P119 DEVELOPMENT OF A POWERED 'NEURO' WHEELCHAIR PRESCRIPTION

ROLFE J[1,2]

[1]*MND Association, Northampton, UK,* [2]*University of Oxford, Oxford, UK*

Email address for correspondence: jennifer.rolfe@ndcn.ox.ac.uk

Keywords: wheelchairs, quality of life, anticipatory equipment provision

Background: Timely provision of wheelchairs for people with MND has a major impact on maintaining quality of life and social participation, but providers are often unable to keep up with the changing needs of people living with MND, resulting in inappropriate equipment and limited usage (1,2). Wheelchair manufacturers currently offer a range of electrically powered indoor-outdoor chairs (EPIOC). In the UK NHS system the particular needs of people living with MND mean that wheelchairs have to be ordered 'off prescription', increasing the overall cost and delayed delivery timescales.

Objectives: To develop an easy to use and accessible prescription form for a range of EPIOC's for people living with progressive neurological illness, to reduce under prescribing, utilize anticipatory prescribing and be cost effective for public health providers.

Methods: In the first phase of the project, three major wheelchair manufactures (Invacare, Sunrise and Ottobock) were invited to discuss the concept of a Powered Neuro Wheelchair prescription form for their existing range EPIOC's suitable for people living with MND. Steering and focus groups, stakeholder days and user questionnaires were used to inform the development of clinical, functional, and operational requirements of the chair. In the second phase of the project, data is being collected on the uptake, usage and success of the Powered Neuro wheelchair, with preliminary data available by November 2014 (reported by manufacturers sales figures and user and provider satisfaction surveys). On the launch of the wheelchair prescriptions, information was provided to wheelchair providers and users by means of presentations at study days and conferences, manufacturer press and promotional materials and a jointly funded information DVD and product comparison document.

Results: Three manufacturers have developed specific Powered Neuro Wheelchair prescriptions for a range of their EPIOCs for use, at an accessible price within a public healthcare system. These prevent under prescribing as they have a minimum specification meeting the ongoing needs of people living with MND and other progressive neurological diseases.

Discussion and conclusion: The Powered Neuro Wheelchair provides the opportunity to select a clinically appropriate wheelchair for people with progressive neurological conditions. The availability of this chair is likely to result in a reduction in waiting time for provision, and facilitates changes to wheelchair accessories as the disease progresses.

Acknowledgements: Motor Neurone Disease Association (UK), The Department of Health, Oxford MND Care and Research Centre, University of Oxford, Invacare, Sunrise, Ottobock

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P120 EVALUATION OF CURRENTLY AVAILABLE NECK COLLARS FOR COMFORT AND IMPACT ON ACTIVITIES OF DAILY LIVING

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

[1]*Lab4Living, Sheffield Hallam University, Sheffield, S Yorks, UK,* [2]*Sheffield Institute for Translational Neuroscience, The University of Sheffield, Sheffield, S Yorks, UK,* [3]*Devices for Dignity, Healthcare Technology Co-operative, Royal Hallamshire Hospital, Sheffield, S Yorks, UK,* [4]*Barnsley NHS, Barnsley, S Yorks, UK,* [5]*Motor Neurone Disease Association, South Yorkshire, UK*

Email address for correspondence: j.langley@shu.ac.uk

Keywords: design, neck collar, comfort

Background: People with Motor Neurone Disease (MND) often develop weak neck muscles, leading to pain, restricted movement and problems with swallowing, breathing and communication. Ideally, a neck collar would help alleviate these. However, neck collars currently available are of limited use for people with MND and often rejected by patients. The Head-Up project is a 2 year study funded by the National Institute for Health Research (NIHR) Invention for Innovation programme with a budget of £400k. The principal aim is to develop a novel neck orthosis for neck weakness that supports the head, whilst allowing freedom to move, without negatively impacting quality of life. Further papers have been submitted outlining the co-design process and clinical evaluation comparing the new design with a list of user needs established in a workshop before the project with people living with MND.

Objectives: To start with the co-design team wished to understand more about the currently available collars with respect to user comfort and impact on Activities of Daily Living (ADLs).

Methods: A comfort assessment study was conducted using healthy volunteers. Four different neck collars were identified based on common usage and distinct design differences (Philadelphia, Headmaster, Apsen and Stro II). Participants wore each collar for a four hour period on different days, separated by at least a week. During each test period the participants recorded locations of pain/discomfort and associated perceptions of relative severity of pain/discomfort using an instrument based on a McGill pain questionnaire and a Likert Scale. They recorded data relating to emotional reactions caused by the collars, impact on specific ADL's and aesthetic considerations using a Likert Scale.

Results: Thirty-five healthy participants completed the comfort assessment protocol for each of the four collars. There was a clear differentiation between the four. It was observed that the more support a collar gives, the less freedom of movement it gives and the less comfortable it is. Three of the collars resulted in frustration overall whilst the fourth was neutral. All collars had some degree of design compromise such that none met all the needs of the users.

Discussion and conclusion: From a design perspective this study gave the design team a clear insight into existing products and their impact on an individual's day-to-day life. Further the study indicated that existing solutions fall short of meeting user needs whether they are functional, clinical or related to quality of life and wellbeing. This reinforced the necessity of this programme and co-design approach, intended to be more sensitive to and inclusive of user needs and priorities.

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P121 HEAD UP: CO-DESIGNING A NOVEL CERVICAL ORTHOSIS FOR MND PATIENTS

REED H[1], STANTON A[1], LANGLEY J[1], SHAW PJ[2], HERON N[3], QUINN A[4], MCDERMOTT CJ[2]

[1]*Sheffield Hallam University, Sheffield, UK,* [2]*Sheffield Institute of Translational Neurology, Sheffield, UK,* [3]*National Institute of Health Research Devices for Dignity Healthcare Technology Co-operative, Sheffield, UK,* [4]*Motor Neurone Disease Association, South Yorkshire, UK*

Email address for correspondence: heath.reed@dfgroup.co.uk

Keywords: co-design, product innovation, assistive products

Background: People diagnosed with Motor Neurone Disease (MND) can develop neck muscle weakness and experience 'head drop' leading to pain, restricted movement, problems with swallowing, breathing and importantly face to face communication. To help alleviate these quality of life problems patients are regularly prescribed either a 'rigid' neck orthosis or soft collar. However, many currently prescribed neck collar and orthotics products offer either limited degrees of head and neck support or limit head movement through full immobilization being primarily designed for a variety of conditions other than MND, from whiplash to more severe cervical head and neck trauma. Neck collars currently available can be of limited use for people with MND and are often rejected by patients.

Objectives: The aim of the Head Up design project was to develop and design an orthosis for neck weakness that supported the head whilst allowing increased degrees of freedom of head movement and that minimise negative impacts on quality of life. In these respects the Head Up project addresses a currently unmet need and this report describes the design steps undertaken, working with patients, health and clinical practitioners, that have brought about a new concept in head and neck support for people living with MND.

Methods: A multidisciplinary research collaboration involving patients / users, designers, engineers, carers, clinical and healthcare professionals, has taken place using co-research and co-design process methodologies that take into account specific needs and desires. In the main these have been delivered in workshop and focus group events in two principal phases of new product development. Firstly, iterative co-creation through user engagement, a process in which early ideas move through stages of investigative prototyping and feedback, and how discoveries have subsequently manifested in product features, and secondly, how pre-production prototypes were built, are how they have been appraised by users in a formal evaluation phase.

Results: Funded by the National Institute for Health Research (NIHR) Invention for Innovation (i4i) program, and delivered by teams from the University of Sheffield (UoS), Sheffield Hallam University (SHU), the Sheffield Institute for Translational Neuroscience (SITraN) and Devices for Dignity (D4D). The research has resulted in a commercially viable new product with distinct USPs over and above current product offerings that include provision of support with movement, asymmetric and task specific support capabilities and scope for individually tailored support both in terms of level of disease progression and anthropometrically.

Discussion and conclusion: This report is design practice led and as such it discusses the value of design involvement in highly specific subject areas, engaging design more deeply, empathetically and early on in the development process. It illustrates how design can build on clinically focused approaches to patient need by asking 'what' as well as 'how' we should be designing, and discusses the role of co-design in helping to shape and direct research itself "…moving design from a problem solving activity to a question asking activity".

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P122 NEW DISEASE MANAGEMENT TOOLS – CERNER ELECTRONIC MEDICAL RECORD DEPLOYMENT OF AMYOTROPHIC LATERAL SCLEROSIS FUNCTIONAL RATING SCALE-REVISED (ALS FRS-R) VALIDATED WITH MOBILE SMARTPHONE (IPHONE/ANDROID) APPLICATION (ALSFRSR-LITE)

BOCKENEK JA[1,7], BROOKS BR[1,6], SANJAK MS[1,2], LUCAS NM[1], SMITH NP[1], NICHOLS MS[1], BELCHER SL[1], LARY C[1], NEMETH J[1], RUSSO PC[1], LANGFORD VL[1], WRIGHT KA[1], HOLSTEN SE[1], WARD AL[1,5], FISCHER MP[1], OPLINGER H[1], BRAVVER EK[1,6], DESAI UG[1,6], STORY SJ[1, 6], BOCKENEK WL[3,6]

[1]*Carolinas Neuromuscular/ALS-MDA Center Carolinas Medical Center Department of Neurology Carolinas Hea[illegible]*

Neurosciences Institute, Charlotte North Carolina, USA, [2]*Department of Kinesiology, University of North Carolina Charlotte, Charlotte North Carolina, USA,* [3]*Department of Physical Medicine and Rehabilitation - Carolinas Rehabilitation, Charlotte North Carolina, USA,* [4]*Department of Internal Medicine Carolinas Medical Center, Charlotte North Carolina, USA,* [5]*Cabbarus College of Health Sciences Occupational Therapy, Concord North Carolina, USA,* [6]*University of North Carolina School of Medicine Charlotte Campus, Charlotte North Carolina, USA,* [7]*North Carolina State University, Raleigh North Carolina, USA*

Email address for correspondence: benjamin.brooks@carolinashealthcare.org

Keywords: ALSFRSR-Lite, electronic medical record, quality improvement

Background: ALS FRS-R assessed at diagnosis and throughout the course of ALS provides information for developing prognosis (1), survival and service needs in ALS patients. ALSFRS-R assessment has been validated via telephony and via web portal. Bland-Altman analysis demonstrate good reliability of face-to-face and test-retest assessments. Internet and smartphone utilization of patient-provided clinical information has improved cost effectiveness of ALS patient management. Deployment of ALSFRS-R to electronic medical records has been slow despite numerous benefits regarding monitoring and improving care for ALS patients.

Objectives: To deploy Cerner EMR version of ALSFRS-R and validate same in ALS multidisciplinary Clinic with Mobile Smartphone (iPhone/Android) Application (ALSFRSR-Lite).

Methods: Standardized version of ALSFRS-R was codified in Cerner EMR and put online beginning April 2014. ALSFRSR was performed by RNs and entered in Cerner EMR online. ALSFRS-R was performed in parallel by coordinator using ALSFRSR-Lite. Correlation analysis by MedCalc Software Belgium.

Results: 82 Cerner EMR ALSFRS-R and ALSFRSR-Lite ALSFRS-R pairs were analyzed. Compared with paper assessment of ALSFRS-R and scanning ALSFRS-R forms into Cerner EMR, online entry demonstrated increased number of completed ALSFRS-R forms compared with paper forms (98 ± 2 vs 92 ± 5), fewer missing item scores (99 ± 1 vs 88 ± 4), fewer total score summations (0 vs 96 ± 2). Patients misidentified gastrostomy status in ALSFRSR-Lite and Cerner EMR compared with paper ALSFRSR-Lite (36 ± 14 vs 2 ± 2 vs 2 ± 2). Patients scored themselves consistently higher on ALSFRSR-Lite than did RN raters (0.8 ± 0.4) .

Discussion and conclusion: Cerner EMR online ALSFRSR assessment showed improved numbers of completed forms, fewer missing item scores, fewer total score summation errors. Patients using ALSFRSR-Lite consistently rated themselves higher than did in-person RNs. Use of online tools permits improved ascertainment of ALS patient ALSFRS-R

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P123 UNDERSTANDING PSYCHO-SOCIAL PROCESSES THAT UNDERPIN HOW PEOPLE WITH ALS MAKE DECISIONS ABOUT CARE

FOLEY G[1], TIMONEN V[1], HARDIMAN O[1,2]

[1]*Trinity College Dublin, Dublin, Ireland,* [2]*Beaumont Hospital, Dublin, Ireland*

Email address for correspondence: foleyg3@tcd.ie

Keywords: healthcare, experiences, decision making

Background: Researchers have explored ALS service users' experiences of healthcare services and their perceptions of care. However, few studies had identified key psycho-social processes that underpin service user engagement with healthcare services in ALS.

Objectives: The objectives of this study were two-fold. First, we sought to pinpoint key contexts that shape how people with ALS make decisions about care. Second, we aimed to identify psycho-social processes that underpin their decision making in care.

Methods: Using qualitative methods (1), 34 people were sampled from the Irish ALS population-based register between September 2011 and August 2012. Variation in participants' experiences was sought to capture the impact of different contexts on their decision making in care. An in-depth qualitative interview was conducted with each participant in their home focused on their healthcare experiences since the onset of ALS. All interviews were audio-recorded and transcribed. Data were analysed using open, axial, and selective coding procedures to identify key psycho-social processes in the data (1).

Results: Participants exerted control in healthcare services because they perceived that they had lost control (or were losing control) over their lives. Exerting control in healthcare included the freedom to accept and decline services. The majority of participants needed time to process the life-altering impact of ALS before they readily engaged with healthcare services. Participants' anxiety about end-of-life care was alleviated when they engaged with healthcare professionals who they trusted and who reassured them about their care. Participants trusted and felt reassured by healthcare professionals who enabled them to be in control of their care. Family, parenthood and life stage were primary contexts to how participants engaged with services. Those with dependent children struggled most to come to terms with loss and they actively engaged with supportive and life-sustaining interventions to continue parenting. Participants in later life were somewhat more accepting of death than young and middle-aged participants because they perceived their children to be self-sufficient. Older adults were less inclined to seek life-sustaining interventions. Those without a spouse/partner and/or children suggested they had more freedom than those with family in making decisions about care.

Discussion and conclusion: Service providers should be attuned to the role of parenthood at different life stages for people with ALS and how parenting impacts on the decision-making process in care. Life-course trajectories shape how people with ALS engage with services. Service providers should afford choice to people with ALS about their care and engage with them when they are ready to engage. People with ALS negotiate loss by engaging with services on their own terms.

Acknowledgements: Funding: Health Research Board (Ireland)

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P124 DISCRETE CHOICE EXPERIMENT FOR PREFERENCES OF CARE IN MOTOR NEURONE PATIENTS AND THEIR CARERS

MAGUIRE S, TOBIN K, NORMAND C, HARDIMAN O

Trinity College Dublin, Dublin, Ireland

Email address for correspondence: smaguire@rcsi.ie

Keywords: preferences, discrete choice experiment, health services

Background: Multidisciplinary care improves survival and quality of life in ALS. However, patient preference has not been fully assessed. Stated preference methods in particular Discrete Choice Experiments (DCE) have been frequently used in healthcare in recent years to study the preferences of patients (1).

Objectives: To design and analyse a DCE to elicit patient and carer preferences around healthcare services and palliative care intervention, in the absence of revealed preference data.

Methods: Patients/carers were asked to state their preference over alternative clinical scenarios/services. Responses are used to determine whether preferences are significantly influenced by the attributes and also their relative importance.

Attributes for the choice experiments were generated through a number of formal discussions with healthcare professionals. These included provision of care, price of services, distance to clinic and waiting times, place of care, patient autonomy, timing of palliative care intervention, personal care arrangements, use of communication technology, availability of phone advice, emotional support and dependence on healthcare professionals. The experiment was piloted with 10 patients and carers to ensure validity and relevance. A fractional factorial design was constructed using R software. A consecutive sample of 29 patients and 22 carers who attended the National ALS Clinic participated in the study. The results were analysed using random effects probit model.

Results: Patients preferred to receive all the information about ALS at the time of diagnosis, rather than when they think they need it ($P = 0.047$) or not at all ($P = 0.021$). Patients preferred that personal care in the home be provided by someone who is not a relative or friend ($P = 0.012$). Referral to palliative care services later in rather than early in the course of the illness ($P = 0.010$). Carers showed a preference toward their loved one receiving regular visits from the community multidisciplinary team and public health nurse rather than having to arrange appointments themselves (significant at the level $P = 0.068$). Carers also preferred to have no emotional support rather than see a counsellor ($P = 0.056$) or go to group sessions with other MND carers ($P = 0.065$).

Conclusion: Using a small convenience sample, the opinions of patients/carers differed regarding service provision. The preferences of patients to maximize the availability of information and delay services that are likely to be of benefit may reflect their preference to retain control throughout the course of their illness, even if at the cost of accessing care and services that are potentially beneficial.(2)

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P125 SOUTH WALES MOTOR NEURONE DISEASE CARE NETWORK – AN AUDIT OF CARE PROVISION IN SOUTH WALES

GLEW R, HANCOCK K, BAKER I, DAWSON K, HADJIKOUTIS S

South Wales MND Care Network, South Wales, UK

Email address for correspondence: ruth.glew@wales.nhs.uk

Keywords: care network, audit, service development

Background: The South Wales Motor Neurone Disease Care Network was established in autumn 2012. It provides an innovative approach to care for people with MND (pwMND) locally through planning, delivery, implementation and evaluation of multidisciplinary teams and clinics within each local health board in South Wales, thus promoting seamless co-ordinated local care. To inform the network development an audit was carried out in January 2013 to discover the issues around care pwMND received.

Objectives: To provide a baseline on which to measure the success of the network approach against MND Association standards of care: to engage pwMND in the new network approach; to identify the needs of pwMND in South Wales and to obtain feedback regarding current services; to ascertain demographic information relating to pwMND.

Methods: A questionnaire consisting of 65 multi- choice and free text questions was sent to the 172 pwMND known to the MND Association and former Cardiff Care Centre. Questions explored all aspects of the patient journey.

Results: Of the 172 questionnaires sent out, 76 questionnaires were returned giving a 44% response rate. 89% of respondents had limb weakness, 56% had bulbar symptoms and 34% had respiratory involvement. 65% were cared for by a spouse or partner but 8% had no carer. PwMND had access to a huge range of equipment but experienced delays in obtaining equipment. 50% of respondents had received MND Association information and 27% had contact with an Association Visitor. 75% had attended a hospital clinic. 85% felt they had had no opportunity to discuss advance care planning. 29% had had an emergency admission to hospital since diagnosis. The attitude of professionals had a big impact on respondents' perceptions of care. Respondents commented on delays in diagnosis and access to care and equipment, lack of communication and change in staff particularly unhelpful. They suggested that improved communication and information, and regular contact with specialist professionals would improve care provision.

Discussion and conclusion: These audit findings have provided an overview of service provision exposing areas of good and not so good practice. The findings have already been used to inform the planning and implementation of the network model and to identify areas requiring service development. The audit will be repeated in January 2015 and subsequent years to close the audit loop and continue to evaluate the impact of the work of the network.

This audit provides a baseline on which to measure improvements in service provision now that the network model is being established and will provide very useful information into the impact of this innovative network approach to care.

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P126 VISION FOR THE FUTURE - THE NETWORK APPROACH: A NEW MODEL FOR CARE PROVISION FOR PEOPLE WITH MOTOR NEURONE DISEASE (MND) - SOUTH WALES MND CARE NETWORK

GLEW R[1], HANCOCK K[1], JAMES J[2], BAKER I[1], DAWSON K[1], HAJIKOUTIS S[1]

[1]*South Wales Motor Neurone Disease Care Network, South Wales, UK,* [2]*Motor Neurone Disease Association, Northampton, UK*

Email address for correspondence: ruth.glew@wales.nhs.uk

Keywords: care network, care, multidisciplinary team

Background: Historically the model for specialist MND patient care provision in the UK has been largely through specialist regional care centres. In South Wales this effectively reached half of the MND population. The new network approach seeks to deliver better more equitable care for people with MND (pwMND) and their families/carers. The model encompasses holistic multi-disciplinary team working with early palliative medicine input at a local level.

Objectives: (i)To highlight the need for a new approach; to inform delegates of our new approach to care provision; (ii) to support professionals within the MND community to provide equity of access to care and care which meets the MND Association Standards of Care.

Aims of network: (i) Development of multi-disciplinary teams (MDT's) and clinics within each local health board to include regular 3 monthly assessment at clinic from Consultant Neurologist, Consultant in Palliative Medicine, respiratory services, care co-ordinator, occupational therapist, physiotherapist, dietician, speech and language therapist, social worker and support from MND Association Visitor. MDT outreach to community for pwMND unable to attend clinic. (ii) Promotion of effective integrated working between health, social service and voluntary sectors. (iii) Improved support and co-ordination of services including training and education.

Results: We now have 4 multi-disciplinary MND clinics established in South Wales, 3 others being established currently and the original Cardiff clinic with associated MDT meetings. Early indications from both pwMND and health and social service staff involved in caring for this patient group show that the network approach is an effective and efficient way of addressing the needs of people with MND and their families.

Patients report feeling more supported and reassured by the presence of a team. They are able to develop a relationship with Palliative Medicine professionals supporting end of life decision making. There are fewer hospital appointments and less disruption to other activities. Team members report better communication between them and report feeling more supported in providing care.

Discussion and conclusion: The poster presentation will outline the South Wales MND Care Network progress to date and is accompanied by a sister poster outlining the processes involved in setting up a network and associated challenges and rewards. By offering 3 monthly assessments within a clinic setting by all professionals involved, problems can be identified early and interventions discussed fully with pwMND and implemented immediately ensuring timely interventions. Providing assessment and care in this way has led to a more co-ordinated approach.

This model of care has benefits for both pwMND and professionals involved in their care. We are presently developing an outcome framework to further evaluate the impact of this approach on health care and patient outcomes.

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P127 VALIDATION OF A NURSING CONTINUING EDUCATION PROGRAM FOR THE CARE OF PATIENTS WITH INTRACTABLE NEUROLOGICAL DISEASE, WITH AN EMPHASIS ON ACTIVE LISTENING

HARA M[1], KONAGAYA M[2], OKADA M[3], TAKEUCHI C[4], MIYAMAE R[5], SHIMODAIRA Y[6]

[1]*Tokyo Women's Medical University School of Nursing, Shinjuku-ku Tokyo metropolitan, Japan,* [2]*School of Nursing and Rehabilitation Sciences Showa University, Yokohama-city, Japan,* [3]*Tokyo Women's Medical University School of Medicine, Shinjuku-ku Tokyo metropolitan, Japan,* [4]*Tokyo Women's Medical University yachio Medical Center, Yachiyo-ctiy Chiba prefecture, Japan,* [5]*NTT Medical Center Tokyo, Shinagawa-ku Tokyo prefecture, Japan,* [6]*Japanese Red Cross Akita College of Nursing, Akita prefecture, Japan*

Email address for correspondence: konagaya@nr.showa-u.ac.jp

Keywords: nursing, education, PBL

Background: Our previous study revealed that nurses experience difficulties in active listening when faced with the ongoing and unforeseen distress and anxiety felt by patients with intractable neurological disease(1).

Objectives: The aim of this study was to validate the efficacy of a nursing continuing education program that would enable nurses to engage in better active listening as a part of the psychological care of patients with intractable neurological disease. Toward this end, the program employed the problem-based learning (PBL) approach (2). Here, we reveal the features of nurses' learning using the PBL approach.

Methods: An educational program comprising three monthly seminars was held for nurses who had cared for patients with intractable neurological disease, and the PBL approach was used in the second and third seminars. Common cases of communication difficulties with patients were recreated and presented. A questionnaire survey was conducted at the end of the program in order to reveal features of the PBL approach. This study was reviewed and approved by the ethics committee of our university.

Results: Analysis of questionnaire results showed that participants derived learning themes, such as "how to encourage patients to engage in conversation", "the meaning of silence", and "preparation for listening to patients who shut others out", through teamwork, thereby experiencing the "successful identification of matters of interest". Nurses gained learning experience, such as "objectively observing how they were listening to patients", "thinking where problems exist and deepening their thoughts", and "perceiving various viewpoints by exchanging opinions", through role-playing and the PBL, thereby identifying clues for addressing communication problems.

Discussion and conclusion: The PBL approach was effective in resolving problems associated with nurses' communication with patients with intractable neurological disease. In particular, role-playing provided nurses with clues to perceive their communication patterns with patients.

Acknowledgements: This study was supported by Grants-in-Aid for Scientific Research (Scientific Research B) from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

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P128 THE SCOTTISH MOTOR NEURON DISEASE AUDIT, RESEARCH AND TRIALS (SMART) STUDY: AN AUDIT OF THE HEALTH CARE OF PEOPLE WITH MND/ALS IN SCOTLAND

SWINGLER R[1,2], GORRIE G[3], DAVENPORT R[1], SALMAN A-S[1], MALIK A[3], HALL G[4], FERNANDES P[1], LIVINGSTONE S[3], PAL S[1], COLVILLE S[1], STEPHENSON L[1], CHANDRAN S[1]

[1]*University Of Edinburgh, Edinburgh, UK,* [2]*NHS Tayside, Dundee, UK,* [3]*Southern General Hospital, Glasgow, UK,* [4]*Aberdeen Royal Infirmary, Aberdeen, UK*

Email address for correspondence: robert.swingler@nhs.net

Keywords: epidemiology, care, standards

Background: In 2005 the Scottish government introduced a programme to improve the care of people with MND/ALS by developing standards for access to services, diagnosis and ongoing care (1,2)

Objectives: We designed the Scottish MND Audit, Research and Trials (SMART) study to provide an independent evaluation of the care of people with MND in Scotland against these standards.

Methods: We ascertained patients added to the SMND Register in 2011 and 2012 (3), and designed a web-based tool to audit medical records retrospectively (4).

- *Access:* We recorded the time from referral to assessment by a neurologist, MND Specialist nurse and a defined MND service.
- *Diagnosis:* We recorded the time from initial consultation to investigation with EMG and MRI, and the time to review with the results.
- *Ongoing care:* We determined whether the health boards of Scotland had an auditable care pathway in place, and reviewed several aspects of care.

Results: In 2013 an interim analysis of 805 patients showed that 97% of patients had access to a neurologist, compared to 80% in 1989-98, and 91% were seen in a defined MND service, but only 75% of patients attended a service providing a full range of care. 51% of patients had access to MRI and diagnostic EMG within 20 working days of initial assessment, and 70% of patients were reviewed within 15 working days of completion of these investigations. Only three out of fourteen health boards (21%) had care pathways in place.

Discussion and conclusion: Qualitative studies indicate that people with MND in Scotland think that access to health services is poor, that the diagnostic process is slow, and that there are deficiencies in long-term care (5). Our quantitative data indicate that access to a neurologist has improved, and most patients are seen in a defined MND service, but the diagnostic process is slow, and few health boards have auditable care pathways.

This is the first national audit of the care of people with MND in Scotland. It shows that access to care has improved, but the diagnostic phase is slow, and we are at present developing tools to assess long-term care more effectively.

Acknowledgements: MND Scotland, The Euan MacDonald Centre for MND Research, participating medical staff

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P129 CAREGIVING EXPERIENCES OF CHILDREN AND ADOLESCENTS OF A FAMILY MEMBER WITH ALS

KAVANAUGH MS[1], BANKER-HORNER L[2], BARKHAUS PE[3]

[1]*University of Wisconsin - Milwaukee, Milwaukee, WI, USA,* [2]*ALS Association, Wisconsin Chapter, Milwaukee, WI, USA,* [3]*Medical College of Wisconsin, Milwaukee, WI, USA*

Email address for correspondence: kavanaug@uwm.edu

Keywords: young carers, quality of life, social support

Background: Caregiving is a critical component of how ALS patients (PALS) survive with a quality of life (QOL). Understanding this caregiving experience is critical for the well-being of both caregivers and PALS. Currently, this is limited to adult caregivers. There are an estimated 1.4 million Young carers (YCs) (< 19 years of age) in the U.S. who provide care to chronically ill family members. (1) To our knowledge, there is no extant data on YCs in ALS. Given the complexity of ALS symptomology and the relatively short duration of disease in many patients, appreciation of the YC's experience may have significant impact on a PALS and their family's QOL.

Objectives: This exploratory study identifies and describes the caregiving experience in a previously unknown population of YCs who have a family member with ALS.

Methods: Semi-structured interviews were conducted with an initial sample of 13 YCs who participate in the care of a family member with ALS. Study participants were accessed through the Wisconsin Chapter of the U.S. ALS Association. Qualitative data was analyzed using thematic content analysis, with descriptive statistics used for quantitative data.

Results: ALS YCs ranged from 8 to 18 years of age. They provide care for both parents (N = 10) and grandparents (N = 3), and spend an average of 10 hours/ week performing tasks including feeding, dressing, and transferring. Sixty-nine percent (N = 9) of YCs stated they had a lot of responsibility. Of those, 5 scored high (13), on the depression inventory. Almost all (N = 12) participants reported they did not have enough information, or support, in dealing with ALS. Qualitative analysis elicited several themes including: (i) caregiving is stressful; (ii) being a caregiver is socially isolating; (iii) caregiving as an opportunity to spend time with their family member with ALS.

Discussion and conclusion: Our findings suggest these YCs for PALS have complex, negative and positive, caregiving experiences. Clearly, they are intricately involved in caregiving, yet receive little acknowledgement or support in that role. Given that social support is vital for the well-being of caregivers, this lack of acknowledgement of the role of YCs in the home can be a source of additional problems for a family struggling with ALS. Results provide clear implications for health care professionals in designing best care practices for PALS, thus lessening the impact on YCs. As more data on YCs becomes available, the results will be used to implement programs, services, and interventions.

Acknowledgements: The study is funded through Helen Bader School of Social Welfare, University of Wisconsin - Milwaukee.

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P130 BEREAVEMENT SUPPORT NEEDS OF FAMILY CARERS OF PEOPLE WITH MOTOR NEURONE DISEASE (MND)

O'BRIEN M[1], KIRKCALDY A[1], JACK B[1], BELL S[2], KNIGHTING K[1], ROE B[1]

[1]*Edge Hill University, Ormskirk, Lancashire, UK,* [2]*Motor Neurone Disease Association, Northampton, UK*

Email address for correspondence: obrienm@edgehill.ac.uk

Keywords: caregivers, bereavement needs, assessment

Background: The importance of assessing and meeting carers' needs during end-of-life care and into bereavement is well documented (1). The bereaved have worse health outcomes than comparable groups of non-bereaved individuals and bereavement is associated with mental health issues, increased risk of suicide and increased healthcare costs (2; 3). Around 10% of bereaved people will develop 'Prolonged Grief Disorder' (PGD) (4) - an unusually intense reaction to the death of a loved one extending beyond the time typically considered normal in bereavement. Little attention has been given to this condition in carers of people with MND.

Objectives: To seek the views of carers and health/social care professionals on bereavement support for carers.

Methods: Two focus groups were conducted with carers (n = 16) and an online survey administered to health/social care professionals in England (MND Care Centre staff, District Nurses and MND Association Regional Care Development Advisors (RCDAs) (n = 59)).

Results: Our focus group data indicate the strain and burden experienced by carers and the need for appropriate support both pre and post-bereavement to be identified and delivered in a timely manner. The survey revealed that health/social care professionals were unsure of the best course of action to take to support carers experiencing bereavement. Forty (68%) respondents suggested that bereavement-related support could be improved; 24 (42%) respondents had encountered family carers of people with MND they considered were suffering from PGD; 38 (68%) respondents did not feel able to accurately predict future cases of PGD and 40 (71%) respondents felt that the implementation of an alert tool to assist in predicting family carers at risk of PGD would be worthwhile.

Discussion and conclusion: Based on our findings bereavement-related support for carers needs to be improved, especially as PGD may be experienced at a higher rate (42%) in this population and we cannot as yet reliably identify the risk of developing PGD amongst carers of people living with MND (plwMND). There is therefore a need to develop an alert system to enable those working with carers of plwMND to identify those carers at risk and subsequently refer them to appropriate support in a timely manner. This is the focus of future work by the team.

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P131 WHY/HOW ARE FAMILIES IN JAPAN ABLE TO CONTINUE PROVIDING LONG-TERM CARE IN SPITE OF THE ASSOCIATED BURDENS?

KAWAGUCHI Y[1], ISHIJIMA K[2], KONAGAYA ME[4], NAKAYAMA Y[3]

[1]*NPO ALS/MND Support Center SAKURA, Tokyo, Japan,* [2]*The University of Tokyo, Tokyo, Japan,* [3]*Laboratory of Nursing Research for Intractable Disease, Tokyo, Japan,* [4]*School of Nursing and Rehabilitation Sciences, Showa University, Tokyo, Japan*

Email address for correspondence: yumiko.kawaguchi@gmail.com

Keywords: burden on family, TPPV, long-term Care

Background: In many countries, most patients do not use Tracheostomy Positive Pressure Ventilation (TPPV), because they do not want to burden their families with long-term care. However, the proportion of Japanese patients who use TPPV is relatively high. Although some researchers have indicated that this ratio is affected by the recommendations of neurologists (1), there has been less empirical research investigating the effect of family members.

Objectives: Our objective is to reveal why/how family members make long-term care possible in Japan despite the associated burdens. This issue is important because our findings can help patients and families in other countries who want to use TPPV but are hesitant to do so.

Methods: Using snowball sampling, we selected 18 children who were providing care to a parent. We focused on children, because they are expected to take care of a patient if his or her spouse continues to work.

We designed a mixed methods research study. First, we quantitatively measured the children's burdens using the Cumulative Fatigue Symptoms Index (CFSI) (2). Then we conducted focus group interviews with them and obtained qualitative data about their long-term care. We analyzed these qualitative data with reference to the CFSI results.

Results: A summary of the CFSI data showed that the children clearly felt burdened. Interview data indicated that this is not only because they have to care for the patients all day but also because the patients sometimes behave abnormally. However, we also found that the children's lives were not completely taken up with long-term care. Some had hobbies such as painting, cooking or photography, for instance, or had obtained paid employment utilizing the skills they had gained through providing long-term care. We also found that when the children were not crushed by the burden of care the patients did not regret using TPPV.

Discussion and conclusion: Of course children do not want their parents to become ill and to have to provide long-term care, but we show that their burdens do not necessarily make long-term care impossible. Even though these children bear a burden, they are able to handle it and allow the patients to live longer. Our findings therefore suggest that patients do not have to give up their desire to live on the grounds of not wanting to burden their families.

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P132 AN AMBULATORY MODEL OF MEDICAL AND SOCIAL CARE FOR MND PATIENTS IN RUSSIA

LYSOGORSKAIA E[1], CHERVYAKOV A[1], FOMINYKH V[1], IVANOVA M[1], VOROBYEVA A[1], ZAKHAROVA M[1], BRYLEV L[2], BYALIK M[3], DIKHTER E[3], FOMINYKH M[3], ORLOVA O[4], SHTABNITSKIY V[3], SONKINA A[5]

[1]*Research Center of Neurology Russian Academy of Medical Sciences, Moscow, Russia,* [2]*Moscow Clinical Hospital 12, Moscow, Russia,* [3]*"Miloserdie" Medical Center, Moscow, Russia,* [4]*Psychological center on Pyatnitskaya street, Moscow, Russia,* [5]*Russian National Research Medical University, Moscow, Russia*

Email address for correspondence: kutakovaev@gmail.com

Keywords: multidisciplinary care, epidemiology, quality of life

Background: According to official statistics there are 3585 cases of MND registered in Russian annually.

Methods: We present the experience of an outpatient program for patients with MND in Russia based on the work of the Martha-Mary Medical Center Miloserdie during 2011–2013 years - the result of collaboration of 11 doctors, 1 psychologist, 2 social workers, 3 nurses, 3 coordinators and 20 volunteers. Our organization provides multidisciplinary care at home, hospitals and with on-line service consultations and work with our patients in the end-of-life decision making process. Our team holds a monthly out-patient clinic. We provide patients with NIV and IV machines and consult on their installation and use. We also organize hospitalizations in local hospitals to perform gastrostomy and tracheostomy. We provide information support through mndfund.ru website.

Results: 250 patients applied to our organization from 20 regions of Russia. 82 of patients died during the studies period. Average patient's age is 58 ± 13 years. 28.4% of patients or their family members applied to out patient clinics. Only 15% of patients take Riluzole because this drug is not registered in the Russian Federation. The first neurologist consultation was provided after 9 months of symptom's onset in average. ALS diagnosis was confirmed in 14 months after disease manifestation.

Only 30% patients have been informed about their diagnosis when they apply for support and treatment to our service. 34% patients had bulbar form of ALS, 66% had spinal form. Mean ALSFRS-R score was 33 ± 6. The large majority of our patients (78%) apply to our service when they have the 4th stage of MND, in particular 31% patients are on 4A stage and 47% - on stage 4B.30% patients use PEG or tube feeding and get enteral nutrition. From the beginning of organization's functioning, 73 patients received NIV machines and 6 received IV. Currently we observe over 37 NIV patients and 7 IV patients. 66% had dyspnea during exertion, daytime sleepiness or morning headaches. 38% patients had pain complaints.

Discussion and conclusion: Our patients had a long time from symptom onset until neurologist consultation, and a long time from consultation to diagnosis establishment. This is not because of slow disease progression but because of the lack of awareness of ALS disease in Russia. Common feature of Russia ALS patients is low level of disease knowledge and education. A main problem is the inability to perform and high cost of NIV and IV machines in Russia, patients do not have full access to pain and anxiety medication, because of strict contraindications in Russia, although we reported high level of pain and dyspnea among our patients. All data concludes that the care for ALS patients in Russia should be reorganized. That was the reason to create our service, and we need to continue our activities.

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THEME 6 EPIDEMIOLOGY

P133 PROFILE OF MEDICAL CARE COSTS IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS IN MEDICARE PROGRAM AND UNDER COMMERCIAL INSURANCE

MENG L[1], JORDAN S[1], BIAN A[1], ANDREW J[1], SHEFNER J[2], WOLFF A[1], BLUMEN H[3], IWASAKI K[3], PYENSON B[3]

[1]*Cytokinetics, Inc., South San Francisco, USA,* [2]*SUNY Upstate Medical University, New York, USA,* [3]*Milliman, Inc, New York, USA*

Email address for correspondence: lmeng@cytokinetics.com

Keywords: cost, Medicare, disability

Background: Amyotrophic lateral sclerosis (ALS) is a rare disease causing progressive denervation and consequent skeletal muscle atrophy leading to weakness, fatigue, muscle weakness and death, primarily from respiratory complications. Treatment consists of modest alteration of disease progression, symptom alleviation, and supportive measures.

Objectives: The primary objective of this analysis was to characterize the medical care costs incurred for ALS patients covered by Medicare and commercial insurance. Specifically, this analysis will determine the ALS medical care costs before and after diagnosis under each program and during the transition between these two programs.

Methods: 368 ALS patients who were diagnosed between January 1, 2009 and December 31, 2010 were identified from a 5% sample of Medicare claims (2008–2011). 344 ALS patients who were diagnosed using the same methodology as the Medicare cases were also selected from the Truven Marketscan commercial database (2008–2010). The monthly claim costs were tabulated from one year before the index date until death or the end of 2011 for Medicare cases. An exponential regression was used to model the monthly cost prior to index date. Kaplan-Meier Sample Average was employed to model the lifetime cost since index date, adjusting for censored cases. Medical costs from index date to disability milestones were tabulated from the Medicare database.

Results: Both Medicare program and commercial insurance demonstrate comparable monthly cost patterns before diagnosis. The cost increased exponentially within approximately 8 months before diagnosis. The monthly claim cost dropped sharply after the diagnosis month and then increased steadily until death. ALS patients initially covered under commercial insurance and then switched to Medicare coverage had comparable patterns to patients in pure Medicare programs. Approximately 30% of ALS patients already received supportive services for disabilities at the time of diagnosis. Total cost incurred in pure Medicare programs increased rapidly and substantially with worsening disability milestones.

Discussion and conclusion: This analysis only includes Medicare and commercial costs. Out of pocket costs likely increase the economic burden significantly. The increasingly important roles of risk adjusters, reinsurance and pooling were not assessed in this analysis. Limited duration of follow-up makes many of our conclusions tentative. Nevertheless, these results provide an initial estimate of the economic burden associated with disability milestones in ALS medical care. It will be important to explore whether delaying time to disability milestones reduces costs, as agents that improve function in ALS patients are developed.

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P134 THE ROLE AND VIEW OF STUDY COORDINATORS IN A MULTICENTER ALS STUDY (ALS COSMOS)

HUPF J[1], SINGLETON J[1], KLINGERMAN S[2], COWARDIN C[3], RODRIGUEZ W[4], HAND S[4], WASHINGTON M[4], GORHAM N[5], LIM S[6], CREGAN M[6], OLALDE H[7], DUONG Y-NY[8], FACTOR-LITVAK P[9], MITSUMOTO H[1]

[1]*Eleanor and Lou Gehrig MDA/ALS Research Center, Columbia University Medical Center, New York, NY, USA,* [2]*Mayo Clinic, Rochester, MN, USA,* [3]*Department of Neurological Sciences, University of Nebraska Medical Center, Omaha, NE, USA,* [4]*Texas Neurology, Dallas, TX, USA,* [5]*Department of Neurology and Neurotherapeutics, University of Texas Southwestern, Dallas, TX, USA,* [6]*Department of Neurology, University of California, Davis, Sacramento, CA, USA,* [7]*Department of Neurology, University of Iowa, Iowa City, IA, USA,* [8]*Department of Neurology, University of California, San Francisco, San Francisco, CA, USA,* [9]*Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY, USA*

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

Keywords: observational studies, recruitment, ALS COSMOS

Background: The successful execution of multicenter observational studies falls largely on study personnel, who coordinate and implement the study protocol at study sites. The roles of study coordinators as protocol implementers and co-investigators in multicenter amyotrophic lateral sclerosis (ALS) studies, and their perspectives on both study implementation and success, have not been previously reported.

Objectives: The purpose of this study was to investigate the role and individual viewpoints of study coordinators in Columbia University Medical Center's Multicenter ALS Cohort Study of Oxidative Stress and Disease Progression (ALS COSMOS). By identifying issues unique to both observational multicenter studies and ALS populations, this investigation aims to improve coordinator performance and overall study execution in future studies.

Methods: A 57-item survey was distributed to 24 active research coordinators (RCs) at 14 ALS COSMOS sites across the United States. Former RCs were also asked to participate. The survey covered the background and responsibilities of RCs, subject recruitment and retention, methods of execution, data management, ways to improve future studies, and general sources of satisfaction and dissatisfaction.

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Results: Among the 17 respondents, the vast majority had extensive RC experience. However, only about half had ever coordinated a multicenter study. All were satisfied with pre-study training, but most agreed that training methods could be improved. Most RCs reported that coordinators had left the project mid-study, and that these departures had noticeable effects on implementation of the project. Almost all respondents noted that recruitment was relatively easy, and that PIs or co-PIs typically led recruiting efforts at patients' first follow-up ALS clinic visits. Notable determinants for decreased recruitment were study commitments being too large and competition from clinical trials. While retention was not an issue for most sites, disease progression was the most common reason for subject drop-out. The majority of those surveyed were satisfied with the study as a whole. Nearly all respondents stated that their overall workloads fell within the range of "just right" to "overworked."

Discussion and conclusion: Although most RCs were satisfied with the project, their responses highlighted areas for improvement. RCs often cited subjects' declining ability to speak, write, and travel to clinic as a factor in attrition rate. Using questionnaires and rating scales designed to accommodate subjects with motor and speech deficits, and offering reimbursement for travel expenses, could facilitate continued participation. To compensate for staffing changes, a web-based training module could ease transitions between RCs, and also allow current RCs to refresh their training as needed. Lastly, more efficient distribution of workload could improve study execution, as many RCs reported feeling overworked.

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P135 SOCIOECONOMIC DIFFERENCES IN FUNCTIONAL PARAMETERS AT ALS DIAGNOSIS

FACTOR-LITVAK P[1], GOETZ R[2], HUPF J[3], SINGLETON J[3], MITSUMOTO H[3], STUDY GROUP ALS COSMOS[3]

[1]*Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, New York, USA,* [2]*New York State Psychiatric Institute and Department of Psychiatry, 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*

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

Keywords: epidemiology, socioeconomic, ALS COSMOS

Background: Little is known regarding characteristics associated with function at diagnosis in ALS patients.

Objective: To evaluate sociodemographic and economic differences in function at diagnosis in ALS-COSMOS, a multi-site study of ALS progression.

Methods: Data were collected at the first clinical evaluation and via telephone questionnaire. Function was measured using the ALSFRS-R and %Forced Vital Capacity (%FVC). Sociodemographic and economic indicators included age, race/ethnic group, sex, education level, total annual income, employment status, and health insurance type. We also evaluated smoking and use of alcoholic beverages. We used linear regression, controlling for patient reported duration of symptoms.

Results: 355 patients were enrolled, of whom 324 and 325 had ALS-FRS and FVC data, respectively. Mean ALS-FRS-R and %FVC (+ standard deviation (SD)) were 36.0 (+ 6.76) and 79.2% (+ 22.6), respectively. Average age was 61.1 (+ 10.3) years. 61% were male, 11% were non-white and 6.5% were Latino. The median duration of symptoms prior to diagnosis was 11.5 months. Controlling for duration of symptoms, lower ALS-FRS-R scores were found among: males ($p = 0.02$); non-whites ($p = 0.017$); and those of Latino descent ($p = 0.012$), with lower education ($p = 0.01$); with incomes <$60,000 per year ($p = 0.013$) or incomes between $60,000 and $99,000 per year ($p = 0.12$); employed part time ($p = 0.10$); retired ($p < 0.001$) or on disability ($p < 0.001$); with government sponsored health insurance ($p = 0.071$) and missing data on alcohol use ($p = 0.003$). When all variables were simultaneously adjusted, lower ALS-FRS-R was associated with: non-white race ($p = 0.044$); Latino descent ($p = 0.002$); being employed part-time ($p = 0.10$); retired ($p = 0.001$); disabled ($p < 0.001$); and longer duration of symptoms ($p = 0.037$). Controlling for duration of symptoms, lower %FVC was associated with non-white race ($p = 0.10$); lower education ($p = 0.004$); part-time employment ($p = 0.089$); retired ($p = 0.001$) or disabled ($p = 0.010$); and having government sponsored insurance ($p = 0.071$). Moderate alcohol consumption was associated with higher %FVC ($p = 0.055$). When all variables were simultaneously adjusted, lower %FVC was associated with older age ($p = 0.027$), low education level ($p = 0.035$), retired ($p = 0.063$) or disabled ($p = 0.021$) and longer duration of symptoms ($p = 0.056$); higher %FVC was associated with moderate alcohol intake ($p = 0.078$).

Discussion and conclusion: Variables reflecting sociodemographic characteristics are associated with functional severity of ALS at enrolment. These relationships are possibly due to general doctors not referring patients to tertiary care clinics at early stages or use of an informal care system (ie, by relatives in the home) for patients with mild to moderate symptoms.

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P136 FACTORS AFFECTING LONGITUDINAL FUNCTIONAL DECLINE AND SURVIVAL IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

WATANABE H[1], ATSUTA N[1], NAKAMURA R[1], HIRAKAWA A[2], WATANABE H[1], ITO M[1], KATSUNO M[1], IZUMIY[3], MORITA M[4], TOMIYAMA H[5], TANIGUCHI A[6], ABE K[7], MIZOGUCHI K[8], KANO O[9], KUWABARA S[10], IMAI T[11], AOKI M[12], KAJI R[3], NAKANO I[13], SOBUE G[1]

[1]*Department of Neurology,* [2]*Biostatistics Section, Center for Advanced Medicine and Clinical Research, Nagoya University Graduate School of Medicine, Nagoya, Japan,* [3]*Department of Clinical Neuroscience, Institute of Health Biosciences, University of Tokushima Graduate School, Tokushima, Japan,* [4]*Division of Neurology, Department of Internal Medicine, Jichi Medical University, Tochigi, Japan,* [5]*Department of Neurology, Juntendo University School of Medicine, Tokyo, Japan,* [6]*Department of Neurology, Mie University Graduate School of Medicine, Tsu, Japan,* [7]*Department of Neurology, Okayama University Graduate School of Medicine, Okayama, Japan,* [8]*Department of Neurology, National Hospital Organization, Shizuoka-Fuji National Hospital, Fujinomiya, Japan,* [9]*Department of Neurology, Toho University Omori Medical Center, Tokyo, Japan,* [10]*Department of*

Neurology, Graduate School of Medicine, Chiba University, Chiba, Japan, [11]*Division of Neurology, National Hospital Organization, Miyagi National Hospital, Miyagi, Japan,* [12]*Department of Neurology, Tohoku University School of Medicine, Sendai, Japan,* [13]*Department of Neurology, Tokyo Metropolitan Neurological Hospital, Tokyo, Japan*

Email address for correspondence: aoimonpi@yahoo.co.jp

Keywords: ALS registry, ALSFRS-R, survival

Background: Functional decline and survival are equally important for evaluating the clinical course of amyotrophic lateral sclerosis (ALS) patients. However, although the factors that affect survival have been well analysed, factors that affect functional decline have not been well demonstrated.

Objectives: The aim of this study was to elucidate the clinical factors affecting functional decline and survival in Japanese ALS patients.

Methods: We constructed a multicentre prospective cohort of ALS patients and included 451 sporadic ALS patients in the analysis. We longitudinally utilized the revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) as the functional scale, and we determined the timing of events such as the introduction of a tracheostomy for positive-pressure ventilation and death in the included patients. We employed a joint modelling approach to identify prognostic factors for functional decline and survival (1, 2).

Results: Age at onset was a common prognostic factor for both functional decline and survival ($p < 0.001$, $p < 0.001$, respectively). Female gender ($p = 0.019$) and initial symptoms, including upper-limb weakness ($p = 0.010$), lower-limb weakness ($p = 0.008$) or bulbar symptoms ($p = 0.005$), were related to early functional decline, whereas neck weakness as an initial symptom ($p = 0.018$), non-use of riluzole ($p = 0.030$) and proximal dominant muscle weakness in the upper extremities ($p = 0.01$) were related to a shorter survival time. A decline in ALSFRS-R score correlated with a shortened survival time ($p < 0.001$).

Discussion and conclusions: The factors affecting functional decline and survival in ALS patients were common in part but different to some extent. This point of difference has not been previously well recognised but is informative in clinical practice and in conducting trials.

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P137 DETECTION OF BETA-N-METHYLAMINO-L-ALANINE IN A LAKE SURROUNDED BY CASES OF AMYOTROPHIC LATERAL SCLEROSIS

STOMMEL E[1], CALLER T[1], HENEGAN P[1], COX P[2], HANEY J[3], METCALF J[2], POWELL J[2], BANACK S[2]

[1]*Neurology Department, Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA,* [2]*Institute for Ethnomedicine, Jackson, WY, USA,* [3]*Department of Biological Sciences, University of New Hampshire, Durham, NH, USA*

Email address for correspondence: patricia.l.henegan@hitchcock.org

Keywords: cyanobacteria, BMAA, environmental risk factor

Background: A cluster of amyotrophic lateral sclerosis (ALS) cases has been previously described bordering Lake Mascoma in Enfield, NH, with an incidence of ALS approximating 25 times that of the baseline incidence of ALS reported throughout most industrialized nations. We hypothesize that the high rate of ALS might be associated with cyanobacterial blooms that have the potential to produce the neurotoxin beta-N-methylamino-L-alanine (BMAA), implicated as the cause of Guam's high rate of ALS (1). Lake Mascoma has a well-established history of blooms and the cyanobacterial liver toxin, microcystin (MC), has been found in abundance as have species of cyanobacteria capable of producing BMAA.

Objectives: To analyze plankton, fish and aerosol samples from Lake Mascoma for the presence of BMAA, the BMAA isomer 2,4-diaminobutyric acid (DAB) and MC.

Methods: Muscle, liver, and brain tissue samples from a Lake Mascoma carp were collected and tested for both free and protein-bound BMAA and DAB (2, 3). Aerosol samples were collected using a portable air sampler and glass fiber filters. Filters were examined with fluorescence microscopy and PCR to detect cyanobacterial presence and analyzed for BMAA, DAB, and MC (4).

Results: In carp brain ($n = 3$) the free BMAA and DAB concentrations measured were 0.03 μg/g ± 0.025 SD and 0.01 μg/g ± 0.002 SD respectively; protein bound BMAA was 0.03 μg/g ± 0.025 SD and protein-bound DAB was not detected (ND). In carp liver ($n = 3$) total BMAA and DAB were 1.28 μg/g ± 0.034 and ND respectively. In carp muscle ($n = 1$) BMAA and DAB were 1.27 μg/g and ND respectively. No cyanobacteria could be detected in the air filters by fluorescence microscopy or PCR analysis for 16S genes using cyanobacterial 16S primers CYA359F and CYA781R. These results suggest that either cyanobacteria were not present, or the biological material had degraded. BMAA was detected in the air filters that underwent a solid phase extraction, as were both BMAA isomers DAB and N-(2-aminoethyl) glycine (AEG). MC in muscle and kidney tissue was ND and in liver (three replicate sets of tissue) were 5.9, 6.3 and 6.3 ng microcystins/g liver wet weight or 59, 63 and 63 ng MC/g liver dry weight.

Conclusions: These results demonstrate that a putative cause for ALS, BMAA is present in an environment that has a documented cluster of ALS. The modes of exposure are discussed as are synergistic mercury toxicity and the implications and potential role for mitigation.

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P138 INCREASED TUMOR NECROSIS FACTOR-A IN THE SKIN OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS–AN IMMUNOHISTOCHEMICAL STUDY

FUJIKURA M, ONO S

Teikyo University Chiba Medical Center, Ichihara, Chiba, Japan

Email address for correspondence: ono@med.teikyo-u.ac.jp

Keywords: immunohistochemistry, skin, tumor necrosis factor-α (TNF- α)

Background: The studies of skin from patients with amyotrophic lateral sclerosis (ALS) have shown unique pathological and biochemical abnormalities. The lack of bedsore formation even in the terminal stages in ALS patients is considered characteristic. Tumor necrosis factor-alpha (TNF-α) is a major inflammatory cytokine that elicits a wide range of biological responses. TNF-α mediates its biological effects through activation of two distinct receptors, TNF-R1 and R2. TNF-α receptors are constitutively expressed on both neurons and glia in the central nervous system (CNS). Several studies have designated TNF-α as a pathogenic mediator in many CNS diseases with an inflammatory component. Although TNF-α can mediate motor neuron death, its role in disease pathogenesis remains unclear. Despite these findings it is unknown whether TNF-α-positive (TNF-alpha+) structures are present in the skin of patients with ALS.

Objectives: To study TNF-α in the skin of ALS patients.

Methods: Skin biopsy specimens were taken from the left biceps from 21 sporadic ALS patients (61.4 ± 9.2 years) and 20 control subjects with other neurologic disorders (63.6 ± 6.7 years). Routine formalin-fixed paraffin-embedded 6 μm sections were immunostained according to standard techniques. A densitometric analysis was performed using an image analysis system.

Results: Immunohistochemistry for TNF-α demonstrated cytoplasmic activity in the epidermis and in some blood vessels and glands. The proportion of TNF-α-positive (TNF-α+) cells in the epidermis in ALS patients was significantly higher ($p < 0.001$) than in controls. There was a significant positive relationship ($r = 0.87$, $p < 0.001$) between the proportion and duration of illness in ALS patients, but there was no such relationship in control subjects. The optical density of TNF-α+ cells in the epidermis in ALS patients was markedly stronger ($p < 0.001$) than in controls. There was a significant positive relation ($r = 0.70$, $p < 0.001$) between the immunoreactivity and duration of illness in ALS patients. In addition, there was an appreciable positive correlation ($r = 0.59$, $p < 0.01$) in ALS patients between the proportion of TNF-α+ cells and the optical density of these cells, but with no correlation in controls.

Discussion and conclusions: The increased TNF-α immunoreactivity of skin does not reflect nutritional status-or activity-dependent skin remodeling. From these considerations, our results as to the increase of TNF-α immunoreactivity of skin in ALS might indicate an augmentation of TNF-α content due to reduced degradation, increased synthesis, and/or increased binding of circulating TNF-α by skin components. These data suggest that changes in TNF-α identified in the skin of ALS patients are likely to be related to the disease process and that metabolic alterations of TNF-α may take place in the skin of patients with ALS.

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P139 WERE NUTRITIONAL FACTORS ASSOCIATED WITH HIGH INCIDENCE OF ALS IN THE K AREA?

OKAMOTO K[1], KIHIRA T[2], KOKUBO Y[3], KUZUHARA S[4]

[1]*Aichi Prefectural University School of Nursing and Health, Nagoya, Japan,* [2]*Kansai University of Health Sciences, Osaka, Japan,* [3]*Graduate School of Regional Innovation Studies, Mie University, Tsu, Japan,* [4]*Suzuka University of Medical Science, Suzuka, Japan*

Email address for correspondence: okamoto@nrs.aichi-pu.ac.jp

Keywords: nutritional factor, epidemiology, K area

Purpose: The aim of this study was to explore nutritional factors to look for the explanatory clues of the high incidence of ALS in the K area from comparisons with the area free from ALS (control area) together with the results of a population-based case-control study conducted in Japan.

Method: A cross-sectional population-based survey was conducted for adults aged ≥ 15 years living in K area and control area in 2003, and for adults aged ≥ 60 years living in O area in 2011. A case-control study was conducted with incident 183 ALS cases and 407 controls. Dietary information was obtained by a self-administered food frequency questionnaire (FFQ), consisting of 97 commonly eaten food and beverage items. Frequency of food consumption was classified into two categories as follows: less frequent ('never/seldom' or 'less than once a week' '1–3 days a week'), and frequent ('4–5 days a week' or 'almost every day'). Dried fish was classified into two categories as follows: less frequent (less than once per week), and frequent ('almost every day'). The differences of means were tested with analysis of variance, and of the distribution of proportions with the chi-square test.

Results: In 2003, the proportion of subjects with frequent intake of dried fish was significantly higher in K area than in control area, and was the highest among the other food consumption. That was also significantly higher in the time of 15 years old than in the time of survey (2012). That in K area (67.9%) was the highest in the area (Aichi cases, Aichi controls, control area, O cho) to have used for the comparison, and was the lowest in Aichi controls (18.7%). In case-control study, higher frequency of dried fish consumption was associated with an increased risk of ALS (1.2 and 1.9, respectively, $p = 0.004$). Frequent intake of dried fish consumption was significantly associated with an increased risk of ALS, even after adjusting for confounding factors (low vs. high, adjusted OR: 4.8 (95%CI; 3.60–10.3)).

Conclusion: The present results suggested that habitual frequent intake of dried fish may have triggered the development of ALS in the K area, given that our case-control study from which time and a place differ also showed similar findings to the K area.

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P140 BMAA ANALYSIS IN THE BRAINS OF AMYOTROPHIC LATERAL SCLEROSIS/ PARKINSONISM-DEMENTIA COMPLEX OF THE KII PENINSULA OF JAPAN

KOKUBO Y[1], BANACK S[2], MORIMOTO S[3], MURAYAMA S[3], TOGASHI T[4], COX PA[2], KUZUHARA S[5]

[1]*Kii ALS/PDC Research Center, Mie University, Graduate School of Regional Innovation Studies, Mie, Japan,* [2]*The Institute for Ethnomedicine, UT, USA,* [3]*Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan,* [4]*Marine Biosystems Research Center, Chiba University, Chiba, Japan,* [5]*Suzuka University of Medical Science, Mie, Japan*

Email address for correspondence: ktyktykty@me.com

Keywords: Kii ALS/PDC, Guam ALS/PDC, BMAA

Background: Amyotrophic lateral sclerosis/parkinsonism-dementia complex of the Kii peninsula of Japan (Kii ALS/PDC) is a unique tauopathy with fronto-temporal dementia. There is a hypothesis that β-N-methylamino-L-alanine (BMAA), which was produced by cyanobacteria, was a trigger for Guam ALS/PDC. The purpose of this study was to examine BMAA in the brains of Kii ALS/PDC.

Objective: Five patients with Kii ALS/PDC (3 ALS, 2 PDC, men 1:4 women, average age: 69.8 years old, average duration of the illness: 7.4 years), two classical ALS and three age-matched normal control were submitted for the study.

Method: To detect BMAA, free and hydrolyzed amino acid extracts from each brain were analyzed using liquid chromatography-tandem mass spectrometry (LC/MS/MS), high-performance liquid chromatography (HPLC), and a Hitachi amino acid analyzer (AAA).

Result: BMAA was detected in ten samples using the HPLC and in six samples using the LC/MS/MS. BMAA was not detected constantly in the brains of Kii ALS/PDC.

Discussion and conclusion: This suggests that the role that BMAA plays as a trigger for Kii ALS/PDC is unclear. Further research is necessary to understand the possible role of BMAA as a trigger in Kii ALS/PDC as the results clearly differ in BMAA content from previously analyzed ALS/PDC brain samples from Guam.

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P141 IS PARENTAL SURVIVAL ASSOCIATED WITH THE RISK OF AMYOTROPHIC LATERAL SCLEROSIS? - EXPLORING THE FITNESS HYPOTHESIS

VISSER AE, SEELEN M, DE GRAAF JA, HULSBERGEN AFC, VELDINK JH, VAN DEN BERG LH

University Medical Center Utrecht, Utrecht, The Netherlands

Email address for correspondence: a.visser-13@umcutrecht.nl

Keywords: fitness theory, parental survival

Background: There is evidence for an association of a higher phenotypic fitness and an elevated risk of amyotrophic lateral sclerosis (ALS). This 'fitness theory' was supported by recent findings that relatives of ALS patients had a reduced risk of cardiovascular diseases.

Objective: To study this fitness hypothesis, we assessed whether parents of ALS patients live longer than parents of controls.

Methods: In a population-based case control study, information regarding age and cause of death of parents was obtained from the Prospective ALS study the Netherlands (PAN) database. Univariate and multivariate Cox regression analysis was used to determine whether survival of the parents of participants was associated with ALS, stratified by mothers and fathers.

Results: 575 sporadic ALS patients and 1082 controls participated in the study. This resulted in data of 1064 parents of patients and 2127 parents of controls. None of the survival analyses were associated with ALS (all p-values >0.05, all HRs 1.02–1.08).

Conclusion: In this population-based case control study no association between the age of death of parents and the risk of ALS has been found. When parental age of death is a reliable proxy for 'familial' cardiovascular fitness, these results do not support the hypothesized fitness theory.

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P142 BLOOD LEAD, BONE TURNOVER, AND SURVIVAL IN AMYOTROPHIC LATERAL SCLEROSIS

FANG F[1], PETERS TL[1,2], BEARD JD[2], UMBACH D[2], KELLER J[3], MARIOSA D[1], ALLEN K[4,5], ODDONE E[5], YE W[1], SANDLER DP[2], SCHMIDT S[5], KAMEL F[2]

[1]*Karolinska Institutet, Stockholm, Sweden,* [2]*National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA,* [3]*Westat, Durham, NC, USA,* [4]*Durham VA Medical Center, Durham, NC, USA,* [5]*Duke University Medical Center, Durham, NC, USA*

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

Keywords: lead, bone turnover, survival

Objectives: To assess the impact of blood lead and bone turnover status on the survival of US military veterans with amyotrophic lateral sclerosis (ALS).

Methods: We conducted a survival analysis among 123 ALS patients enrolled in the National Registry of Veterans with ALS during January-September 2007. Patients were followed from date of diagnosis to death or July 25, 2013. The association of blood lead and plasma biomarkers of bone formation (PINP) and bone resorption (CTX) with ALS survival were estimated by hazard ratios (HRs) derived from Cox models after adjustment for age at diagnosis, site of symptom onset, ALS functional rating scale, and diagnostic delay. Blood lead, plasma CTX and PINP were mutually adjusted for one another.

Results: By the end of study, 93 of the ALS patients (75.6%) had died. In the fully adjusted model, a one-unit increment in $\log_2$-transformed blood lead (ie, a doubling of lead concentration) was associated with a 1.59-fold risk of death (95% confidence interval (CI): 1.11–2.26) among the ALS patients. Similarly, a doubling of plasma CTX concentration was associated with a 1.66-fold risk of death (95% CI: 1.11–2.46). On

the contrary, a doubling of plasma PINP concentration was associated with a 0.56-fold risk of death (95% CI: 0.39–0.80). No clear interactions were observed between these biomarkers and other ALS prognostic indicators.

Discussion and conclusion: Our results suggest that blood lead and plasma bone turnover biomarkers are independent predictors of ALS prognosis. Moreover, given the ease of measurement, bone turnover status may be of special clinical interest.

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P143 APPLICATION OF A STAGING SYSTEM TO THE PROACT DATABASE POPULATION

HEIMAN-PATTERSON T, DEY S, ALEXANDER G, DEBOO A

Drexel University College of Medicine, Philadelphia, Pa, USA

Email address for correspondence: theiman@drexelmed.edu

Keywords: staging, survival, PRO-ACT database

Background: Validated staging systems for ALS can increase understanding of the disease prognosis, help with resource management and research design. Recently, a staging system was proposed for ALS in a UK population (1) using clinical milestones for disease progression. Milestones were defined as: symptom onset in one region (bulbar, upper limb, lower limb or diaphragmatic) (Stage 1); diagnosis (2a); involvement of a second region (2b); third region (3); gastrostomy (4a); non-invasive ventilation(4b). These stages/milestones occurred at predictable times when examined over the course of the illness with Stage 2a at 35%; stage 2b at 38%; stage 3 at 61%; 4a at 77% ; 4b at 80%. To date this staging system has not been validated in any other populations.

Objectives: To validate this proposed staging system in the Pooled Resource Open-Access ALS Clinical Trials (PRO-ACT) database, a multi-national de-identified ALS database.

Methods: We reviewed 8672 cases within the PRO-ACT database and identified 100 subjects with data spanning diagnosis to death in order to compare the stage durations between a global population and the UK population. We identified the percentage of time in each stage for both bulbar and limb onset, demographics and survivals in order to compare the results of the two populations. Survival analysis included Kaplan-Meier plots and results between the PRO-ACT subjects and UK subjects were analyzed with t-tests using the means and sd.

Results: The PRO-ACT subjects included 66 limb onset (24 F, 42M), and 34 bulbar onset (20F, 14M). The average age of onset overall was 58.25 years(Y) (60.5 Y F, 55Y M), 56.7Y (51.6Y M, 60Y F) for bulbar onset and 59.8Y (58.7Y M, 61.8Y F) for limb onset. For PRO-ACT subjects 2a occurred at 29%, 2b at 55%, 3 at 76 %, 4a at 75 % and 4b at 82 % through the disease course. When compared to the UK population, 2a occurred earlier in the disease course ($p = 0.0007$). Also more time was spent between stages 2a and 2b in the PRO-ACT subjects ($p < 0.0001$); but by stage 4 (a and b): there was no significant difference with both populations having gone through 75% of their disease course ($p > 0.05$). Overall, survival was similar in both.

Conclusion: The clinical staging system was easily applied to the global ALS population represented in the PRO-ACT database and although there were differences in early stage disease, both populations had gone through about 75% of the disease course by Stage 4a and 4b. While further validation in prospective studies will be needed, we agree that this staging system holds promise in guiding prognosis, use of resources and clinical research.

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P144 AGE-PERIOD-COHORT ANALYSIS OF TRENDS IN ALS INCIDENCE

TOBIN K, HARDIMAN O

Trinity College Dublin, Dublin, Ireland

Email address for correspondence: tobink2@tcd.ie

Keywords: age-period-cohort, incidence, partial least squares regression

Background: Many studies have shown an increase in rates of incidence and death from ALS over time (1,2). Investigation of age-period-cohort effects is often used to explain temporal trends in incidence. A number of methodological approaches exist for this type of analysis, each with its own set of assumptions and constraints. Here we apply the novel approach of partial least squares regression (PLSR).

Objective: To look at the effects of age, time of diagnosis and birth cohort to explore trends in incidence using PLSR.

Methods: Estimating the distinct impact of age, period and cohort effects on changes to an outcome such as incidence is a frequently discussed issue in the analysis of healthcare trends. By definition, these three variables are perfectly correlated (age + cohort = period) which leads to the well-known *identification problem* in linear models. Traditional regression methods cannot be used with these three covariates without imposing additional constraints, which may be unreasonable or difficult to interpret. The PLSR model overcomes the identification problem and facilitates the estimation of the relative effects of age, period and cohort on the incidence of ALS. Our dataset is a national population based register of ALS.

Results: As expected, analysis showed a significant age effect with incidence of ALS increasing with age. No significant association was found between year of diagnosis and incidence (period effect). The non-linear PLSR model showed a significant cohort effect with increased incidence for those born between 1912–1916 (Coefficient: 0.32, CI: 0.17–0.46), 1927–1931 (Coefficient: 0.43, CI: 0.24–0.62) and 1932–1936 (Coefficient: 0.35, CI: 0.21–0.49).

Discussion and conclusion: Our analysis shows a significant cohort effect on incidence in this population. Further research will be necessary to explore the potential cause of this trend. Possible explanations may be excess exposure to some environmental risk factor in early life, or some unknown workplace hazard.

Conclusion: Through the use of PLSR modelling it is possible to analyse age-period-cohort effects simultaneously and estimate their relative impact on ALS incidence over time (3). These results can be used in planning future studies on the cause of ALS.

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P145 AGGREGATION OF NEUROPSYCHIATRIC DISEASE IN AMYOTROPHIC LATERAL SCLEROSIS KINDREDS: EVIDENCE OF CLUSTERING WITHIN FAMILIES.

O'BRIEN M[1,2], HEVERIN M[2], BYRNE S[2], ELAMIN M[1,2], HARDIMAN O[1,2]

[1]*Department of Neurology, Beaumont Hospital, Beaumont, Dublin, Ireland,* [2]*Academic Neurology Unit, Trinity College Dublin, Dublin, Ireland*

Email address for correspondence: mgtobrien@hotmail.com

Keywords: neuropsychiatry, C9ORF72, genetics

Background: Amyotrophic lateral sclerosis (ALS) is known to be associated with frontotemporal dementia in 14% of cases. A wider spectrum of neurodegenerative and neuropsychiatric disease is increasingly recognized in association with ALS (1).

Aim: To determine whether psychiatric conditions occur with greater frequency within individual ALS kindreds.

Methods: Data from a previously published family history study was interrogated. The study also included a further 138 ALS patients diagnosed from 2012, with age and gender matched controls. Kindreds from ALS patients and controls in which at least one first degree relative was reported to have a neuropsychiatric illness were identified.

Results: Extensive family histories from 302 ALS patients, encompassing 5838 first or second degree relatives, and 221 controls encompassing 5950 first/second degree relatives were included. 119 (36%) of ALS kindreds and 51 (23%) of control kindreds had at least one first or second degree relatives with a history of schizophrenia, psychosis, suicide, depression, alcoholism or autism ($p < 0.005$). 12.6% (38) of ALS kindreds and 5.4% (12) of control kindreds had 3 or more family members with neuropsychiatric illness ($p < 0.004$). Conditions that were over-represented in the ALS kindreds included schizophrenia ($n = 24$, $p = 0.01$), suicide ($n = 30$, $p = 0.001$), autism ($n = 12$, $p = 0.02$). No differences in size of kindred/number of first vs second degree relatives was observed between ALS and control families.

C9ORF72 repeat expansion data was available for 186 (62%) ALS patients. 13% of ALS patients with a family history of neuropsychiatric disease had the *C9ORF72* repeat expansion.

Conclusion: Aggregation of neuropsychiatric disease within ALS kindreds is driven by over-representation in individual kindreds. While the *C9ORF72* repeat expansion accounts for a proportion of these kindreds, additional families negative for the *C9ORF72* repeat expansion also exhibit a strong neuropsychiatric signal suggesting the presence of at least one addition gene associated with both ALS and neuropsychiatric disease in the Irish population.

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P146 CLUSTER ANALYSIS OF ALS RISK IN IRELAND

ROONEY J[1], HEVERIN M[1], VAJDA A[1], CRAMPSIE A[2], TOBIN K[1], BYRNE S[1], MCLAUGHLIN R[1], STAINES A[3], HARDIMAN O[1, 4]

[1]*Trinity Biomedical Sciences Institute, Trinity College, Dublin, Ireland,* [2]*School of Geography, Planning and Environmental Policy, University College Dublin, Dublin, Ireland,* [3]*School of Nursing and Human Sciences, Dublin City University, Dublin, Ireland,* [4]*Beaumont Hospital, Dublin, Ireland*

Email address for correspondence: jrooney@rcsi.ie

Keywords: Spatial epidemiology, cluster analysis, relative risk

Background: We have recently conducted a Bayesian spatial risk analysis of prospectively gathered data of incident cases over a 17.5-year period (1).

Objectives: To perform formal cluster analysis of the Irish ALS population based Register.

Methods: 1,693 cases of ALS in Ireland from January 1995 to December 2013 were identified from the Irish ALS register. Census data were used to calculate an average population for the period and standardized incidence rates (SIRs) were calculated for 3,355 areas based on Electoral Divisions. Bayesian conditional auto-regression was applied to produce smoothed relative risks (RR). SaTScan was run using a Poisson probability based discrete scan statistic to detect clusters of statistically high risk, and SaTScan to identify areas of low risk.

Results: Smoothed maps revealed no overall geographical pattern to ALS incidence in Ireland, although several areas of localized increased risk were identified, and two larger areas, in Clare and Kilkenny, showed lower RR. Formal cluster analysis confirmed these two low risk areas as statistically significant clusters: Clare (Observed: 0 Expected: 13.5, $RR = 0.0$, $P = 0.025$); Kilkenny (Observed: 57 Expected: 105, $RR = 0.53$, $P = 0.015$). None of the areas identified by Bayesian smoothing to have localized high risk were identified as significant high-risk clusters using current methodology.

Discussion and conclusion: The absence of significant high-risk clusters in the republic of Ireland contrasts previous findings of significant high-risk clusters by others (2, 3, 4). The finding of statistically significant low risk areas has not been previously reported for ALS in European countries. We postulate that historical differences in local genetic admixture may account for these findings. Further studies are underway to investigate possible associations between ALS risk and genetic epidemiology.

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P147 ATXN2POLYQ INTERMEDIATE REPEAT IS A MODIFIER OF ALS PHENOTYPE IN A POPULATION BASED STUDY

CALVO A[1,2], MOGLIA C[1], CANOSA A[1,9], BRUNETTI M[3], BARBERIS M[3], RESTAGNO G[3], CONTE A[4], BISOGNI G[4], MARANGI G[5], MONCADA A[5], LATTANTE S[5], ZOLLINO M[5], SABATELLI M[4], BAGAROTTI A[6], CORRADO L[6], MORA G[7], BERSANO E[8], MAZZINI L[8], D'ALFONSO S[6], CHIÒ A[1,2]

[1]*ALS Center, 'Rita Levi Montalcini' Department of Neuroscience, University of Torino, Torino, Italy,* [2]*Azienda Ospedaliero Universitaria Città della Salute e della Scienza, Torino, Italy,* [3]*Laboratory of Molecular Genetics, Azienda Ospedaliero Universitaria Città della Salute e della Scienza di Torino, Torino, Italy,* [4]*Neurological Institute, Catholic University of the Sacred Heart, Roma, Italy,* [5]*Institute of Medical Genetics, Catholic University of the Sacred Heart, Roma, Italy,* [6]*Department of Health Sciences, Interdisciplinary Research Center of Autoimmune Diseases (IRCAD), 'Amedeo Avogadro' University of Eastern Piedmont, Novara, Italy,* [7]*Salvatore Maugeri Foundation, IRCSS; Scientific Institute of Milano, Milano, Italy,* [8]*Department of Neurology, 'Amedeo Avogadro' University of Eastern Piedmont and Azienda Ospedaliera Universitaria Maggiore della Carità, Novara, Italy,* [9]*University of Genova, Genova, Italy*

Email address for correspondence: andreacalvo@hotmail.com

Keywords: ataxin 2(ATXN2), phenotype, population-based study

Background: An intermediate-length (CAG) expansion (encoding 27–33 glutamines, polyQ) in *ataxin 2* (*ATXN2*) gene, already known as the cause of spinocerebellar ataxia type 2 (SCA2), has been found to be related to an increased risk of developing ALS. The clinical characteristics of patients with this expansion remain poorly investigated.

Objectives: The aim of this study was to analyze the frequency of intermediate polyQ expansion in the *ATXN2* gene in a population-based cohort of Italian patients (discovery cohort), with an in-depth assessment of their clinical and prognostic characteristics, and to replicate the findings in an independent cohort of consecutive patients from an ALS tertiary center (validation cohort). We assessed survival of patients with *ATXN2* polyQ expansion compared with patients with normal length expansion.

Methods: PolyQ expansions were assessed in 672 patients incident in Piemonte and Valle d'Aosta regions, Italy, during the 6-year period from 2007 to 2012 (discovery cohort); controls were 509 neurologically healthy subjects, resident in the study area, age- and gender-matched to cases. The validation cohort includes 661 patients consecutively admitted between 2001and 2013 in the ALS Clinic Centre of the Catholic University in Rome, Italy. Diagnostic criteria were identical to those of the discovery cohort. Genomic DNA was isolated from peripheral blood lymphocytes using a standard protocol. The ATXN-2 CAG repeat in exon 1 was amplified using a fluorescent primer and sized by capillary electrophoresis on an ABI 3130 genetic analyzer. We considered as cut-off a repeat size ≥ 31. All ALS cases of both cohorts were also tested for *SOD1*, *TARDBP*, *FUS*, *ANG* and *C9ORF72*. Familial ALS patients were also tested for *OPTN*.

Results: In the discovery cohort the frequency of ≥ 31 polyQ *ATNX2* repeats was significantly more common in ALS cases (19 patients vs. 1 control, $p = 0.0001$; odds ratio 14.8, 95% confidence interval, 1.9–110.8). Patients with an increased number of polyQ repeats have a shorter survival than those with < 31 repeats (median survival, polyQ ≥ 31, 1.8 years, interquartile range (IQR) 1.3–2.2; polyQ < 31, 2.7 years, IQR 1.6–5.1) ($p = 0.001$). An increased number of polyQ repeats remained independently significant also in multivariable analysis. In the validation cohort, patients with ≥ 31polyQ repeats have a shorter survival than those with < 31 repeats (median survival, polyQ ≥ 31, 2.0 years, IQR 1.5–3.4; polyQ < 31, 3.2 years, IQR 2.0–6.4; $p = 0.007$).

Conclusions: We have found that an *ATXN2* intermediate polyQ repeat is a significant risk factor for ALS; it is correlated to a spinal phenotype and is associated to a shorter survival. Disease-modifying therapies targeted to *ATXN2* represent a promising therapeutic approach for a devastating disease such as ALS; possible strategies may be the use of antisense oligonucleotides, transcription activator-like effector nucleases, and clustered regularly interspaced short palindromic repeats.

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P148 PROGNOSTIC FACTORS FOR ALS IN PATIENTS FROM EMILIA ROMAGNA, ITALY: A POPULATION BASED STUDY

MANDRIOLI J[1], GEORGOULOPOULOU E[1], FINI N[1], BIGUZZI S[2], SETTE E[3], SALVI F[4], PIETRINI V[5], GUIDI C[6], TERLIZZI E[7], RIZZI R[8], CASMIRO M[9], LIGUORI R[10], RAVASIO A[11], D'ALESSANDRO R[12]

[1]*Department of Neuroscience, St. Agostino-Estense Hospital, MODENA, Italy,* [2]*Department of Neurology, Bufalini Hospital, CESENA, Italy,* [3]*Department of Neurology, St. Anna Hospital, FERRARA, Italy,* [4]*Department of Neurology and IRCCS Istituto delle Scienze Neurologiche di Bologna, BOLOGNA, Italy,* [5]*Department of Neuroscience, University of Parma, PARMA, Italy,* [6]*Department of Neurology, Forlì Hospital, FORLI', Italy,* [7]*Department of Neurology, G. Da Saliceto Hospital, PIACENZA, Italy,* [8]*Department of Neurology, IRCCS Arcispedale Santa Maria Nuova, REGGIO EMILIA, Italy,* [9]*Department of Neurology, Faenza and Ravenna Hospital, RAVENNA, Italy,* [10]*Department of Biomedical and Neuromotor Sciences, and IRCCS Istituto delle Scienze Neurologiche di Bologna, BOLOGNA, Italy,* [11]*Department of Neurology, Infermi Hospital, RIMINI, Italy,* [12]*Neuroepidemiology Unit, IRCCS Istituto delle Scienze Neurologiche di Bologna, BOLOGNA, Italy*

Email address for correspondence: J.mandrioli@ausl.mo.it

Keywords: population based registry, survival, prognostic factors

Background: Although the mean survival of ALS patients from symptom onset is often reported to be 3–5 years, published studies report a wide range of outcomes, that narrows when considering population based studies (median survival from onset to death: 20–36 months).We performed a population-based study focusing on ALS survival and possible prognostic factors.

Methods: From 2009 onwards, a prospective registry, involving 9 provinces, 11 local health units, and 17 neurological departments, has been collecting all cases of incident ALS among residents in Emilia Romagna region (population 4.4 million inhabitants). For each patient, the main demographic and clinical information were collected by the caring physicians and input in a dedicated website at diagnosis. In addition a further case report form has been completed during each patient follow up. Clinical characteristics, services access, accepted procedures, and date of tracheostomy and death have been included in the follow up case report form.

Results: From 1.1.2009 to 31.12.2013 in Emilia Romagna 566 patients (M: F = 1.2) received a new diagnosis of ALS. Mean age at onset was 66.4 years. Median overall tracheostomy-free survival from onset was 50 months, 37 months from diagnosis. Based on univariate analysis, factors related to survival were: age at onset, site of onset, diagnostic delay, degree of diagnostic certainty according to El Escorial Revised Criteria (EEC-R), and dementia. In the Cox-multivariable model, the factors independently related to a longer survival were age at onset (HR 1.3, $p < 0.01$), dementia (HR 1.4, $p < 0.01$), respiratory onset (HR 2.4, $p = 0.01$), definite ALS at diagnosis according to EEC-R (HR 1.9, $p < 0.01$), clinically probable ALS at diagnosis according to EEC-R (HR 1.5, $p < 0.01$).

Discussion and conclusions: This population based study confirms the well-known prognostic role of clinical features, such as age at onset, respiratory onset, and dementia together with the degree of certainty of diagnosis (the more certain, the worse disease course). Procedures did not result in disease prolongation, possibly in relation to the greater disease severity in patients requiring respiratory or nutritional support. These confirmatory findings provide evidence that population-based studies are the most reliable settings for epidemiological and clinical studies on ALS.

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P149 EPIDEMIOLOGY OF AMYOTROPHIC LATERAL SCLEROSIS IN ISRAEL (1997–2013)

WEIL C[1], SHALEV V[1,2], HERZEL E[1], CHODICK G[1, 2]

[1]*Maccabi Healthcare Services, Tel Aviv, Israel,* [2]*Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel*

Email address for correspondence: weil_c@mac.org.il

Keywords: prevalence, incidence, survival

Background: Globally, the incidence and prevalence of amyotrophic lateral sclerosis (ALS, or motor neurone disease) are estimated at 1.9 and 4.5 per 100,000 population, respectively (1). There is a need for real-world data on the epidemiology of ALS/MND in Israel.

Objectives: We aimed to determine the prevalence and incidence of ALS/MND in a large Israeli health maintenance organization, and to describe patient characteristics and survival.

Methods: The study was conducted using the computerized databases of Maccabi Healthcare Services, which include up to 20 years of data on 2 million members (25% of the Israeli population). Patients with ALS were identified by diagnosis code (ICD-9 335.2) from a GP or relevant specialist. Prevalence and incidence rates were adjusted to the age distribution of the WHO standard population. Kaplan-Meyer analysis was used to assess patient survival during the period 1997–2013. Demographic and clinical characteristics of prevalent patients (2013) were compared to a control group of ALS-free MHS members matched (1:5) on age (± 5 y) and sex.

Results: Between 1997 and 2013, a total of 456 MHS members were diagnosed with ALS, corresponding to an age-adjusted incidence rate (per 100,000) of 1.7 (95% CI: 1.6–1.8). In 2013 (n = 226), the age-adjusted prevalence rate (per 100,000) was 10.9 (95% CI: 9.5–12.5). The mean age was 58.1 ± 17.5 (59% male). Compared to the control group (n = 1130), patients with ALS had a lower proportion of chronic conditions, such as overweight (26.1% vs.34.0%; $p = 0.024$), diabetes (12.4 vs. 18.0%; $p = 0.042$) and CKD (11.9 vs. 17.0%, $p = 0.060$). During the total follow-up period, 224 (49.0%) of patients with ALS died; the median survival time was 4.6 years (95% CI: 3.3–5.9 y). Approximately 40% of patients survived for at least 8 years after diagnosis and more that 20% survived beyond 10 years.

Discussion and conclusion: The results of this large population-based study indicate that the prevalence of ALS in Israel is relatively high, while the incidence rate is similar to previous international reports (1). One explanation may be relatively higher survival rates for patients with ALS in Israel compared to Western countries. In line with previous studies, the present study suggests that patients with ALS may have a lower prevalence of chronic comorbidities than the general population (2–3).

Conclusion: Based on the present study, there were at least 750 patients living with ALS in Israel at the end of 2013. Further research is needed to investigate factors that may contribute to the survival of patients with ALS in Israel.

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P150 THE LONG SAGA OF A VCP GENE MUTATION IN A LARGE FAMILY

BLUMEN S[1,2], GONZALES-PEREZ P[3], DRORY V[4,5], BROWN JRR[3]

[1]*Hillel Yaffe Medical Center, Hadera, Israel,* [2]*Rappaport Faculty of Medicine, the Technion, Haifa, Israel,* [3]*University of Massachusetts, Worcester, MA, USA,* [4]*Tel-Aviv Sourasky Medical Center, Tel Aviv, Israel,* [5]*Tel Aviv University, Tel Aviv, Israel*

Email address for correspondence: navabl@hotmail.com

Keywords: valosin-containing protein (VCP), myopathy, bulbar

Background: Mutations in the valosin-containing protein (VCP) gene may produce autosomal dominant inclusion body myopathy (IBM), Paget disease of the bone, frontotemporal dementia and familial ALS in different combinations (1).

Objectives: To present the long term follow-up of a large, three generation family with "pseudo-myopathic" ALS and the diagnostic challenges preceding the final identification of a novel, causative, VCP mutation (2).

Family Study: We followed for 13 years a large Arab - Israeli family in which seven members, in three generations, presented sequentially with a complex, slowly progressive neu-

rological phenotype. Since adolescence affected individuals had insidious onset of nasal voice. However, at presentation, at the age of 38, most features in the index patient and his siblings suggested primary muscle involvement: hyperlordosis, proximal weakness, involvement of thoracic paraspinal muscles and moderately elevated CPK. Motor and sensory nerve conduction studies were normal and EMG displayed both neuropathic and myopathic features. At that stage a quadriceps muscle biopsy was not conclusive. Only four years later, proximal and distal muscle wasting with fasciculations, new bulbar symptoms (mild dysphagia) and pyramidal signs in four limbs suggested motor neuron disease. Pompe disease, hexosaminidase A deficiency and SOD1 mutations were excluded. After more than seven years from presentation four brothers (from the second generation) required wheelchairs and, one up to three years later, three among them required respiratory assistance. The index patient is alive on a respirator at 13 years since presentation while two of his brothers died at eight and ten years from presentation. Two patients developed also Parkinsonian features. Cognition and affect were normal in all. The final diagnosis of a point mutation (p.R191G) in the VCP gene was possible only with genetic linkage analysis and whole exome sequencing in two affected brothers (2).

Discussion and conclusion: The long term follow-up of this large family exemplifies the difficulties in discriminating between primary muscle and motor neuron diseases in certain, complex, ALS phenotypes. It also shows that early bulbar features do not always predict a rapidly progressive disease. Finally, our experience stresses the importance of whole exome sequencing as an invaluable diagnostic tool for unusual phenotypes in large families.

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P151 THE GERMAN PRE-SYMPTOMATIC ALS RISK-CARRIER STUDY (GPS-ALS) 2014

WEYDT P, MADINGER M, KNEHR A, BÖHM S, GÖRGES M, KASSUBEK J, WEISHAUPT JH, LUDOLPH AC

Ulm University, Ulm, Germany

Email address for correspondence: patrick.weydt@uni-ulm.de

Keywords: presymptomatic, familial ALS, gene carrier

Background: Recent advances in the understanding of genetic factors underlying familial and sporadic ALS creates important new challenges and opportunities for both the patient community and the clinical and basic researchers. Through genetic testing of symptomatic ALS patients with and without a family history of the disease, first degree relatives can be identified that hold a 50% risk of carrying an ALS-causing gene. Clinical characterization of these presymptomatic risk- carriers offers the possibility to study the earliest, subclinical phases of the disease and thus identifying biomarkers and critical therapeutic intervention points.

Methods: ALS-gene risk carriers are recruited from first degree relatives of ALS patients with known gene mutations (C9ORF72, SOD1, FUS, TDP-43 and others). Regardless of the actual gene status the risk carriers are offered a panel of annual exams consisting of a clinical exam, tissue collection (blood, CSF and others), imaging studies, metabolic test and cognitive exams. The participants and the examiners are blinded to the gene status. Non-mutation carriers serve as internal controls. Genetic counselling is offered to interested parties. A structured phone interview 1 week after the enrolment is used to assess psychosocial effects of the study participation.

Results: >30 participants have been enrolled. As expected, C9ORF72 and SOD1 are the most common ALS associated mutations in our cohort. Acceptance of the individual test was high (>90%), except lumbar puncture (>50%). The data and biosamples are being made available to researchers looking for wet and dry biomarkers.

Discussion and conclusion: This ongoing study offers the opportunity to investigate the early, presymptomatic (prodromal) phase of ALS while at the same time empowering the growing community of people aware of their risk of developing ALS to participate in the quest for a cure.

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P152 THE EPIDEMIOLOGY AND TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS IN CANADA: NEW INSIGHTS FROM THE CANADIAN NEUROMUSCULAR DISEASE REGISTRY

HEGEDUS J[1], KORNGUT L[1,2], ZINMAN L[2,3], JOHNSTON M[1,2], BENSTEAD T[2,4], BRIEMBERG H[2,5], DUPRÉ N[2,6], GENGE A[2,7], GRANT I[2,4], HADER W[2,8], JOHNSTON W[2,9], KALRA S[2,9], MASSIE R[2,7], MELANSON M[2,10], O'CONNELL C[2,11], SCHELLENBERG K[2,12], SHOESMITH C[2,13], WEE J[2,10], WORLEY S[2,10]

[1]*Calgary ALS and Motor Neuron Disease Clinic, South Health Campus, Department of Clinical Neurosciences, Hotchkiss Brain Institute, University of Calgary, Calgary, Alberta, Canada,* [2]*Canadian Neuromuscular Disease Registry Investigator Network, Calgary, Canada,* [3]*ALS Clinic, Sunnybrook Health Sciences Centre, University of Toronto, Toronto, Ontario, Canada,* [4]*Division of Neurology, Dalhousie University, Halifax, Nova Scotia, Canada,* [5]*Division of Neurology, University of British Columbia, Vancouver, British Columbia, Canada,* [6]*Department of Neurological Science CHA-Enfant-Jésus, Laval University, Quebec City, Quebec, Canada,* [7]*ALS Program, Montreal Neurological Institute, Montreal, Quebec, Canada,* [8]*Department of Physical Medicine and Rehabilitation, University of Saskatchewan, Saskatoon, Saskatchewan, Canada,* [9]*Division of Neurology, University of Alberta, Edmonton, Alberta, Canada,* [10]*Departments of Medicine and Physical Medicine and Rehabilitation, Queen's University, Kingston, Ontario, Canada,* [11]*Stan Cassidy Centre for Rehabilitation, Fredericton, New Brunswick, Canada,* [12]*Section of Neurology, University of Manitoba, Winnipeg, Manitoba, Canada,* [13]*Clinical Neurological Sciences, Western University, London, Ontario, Canada*

Email address for correspondence: janka.hegedus@gmail.com

Keywords: registry, epidemiology, Canada

Background: The Canadian Neuromuscular Disease Registry (CNDR) was launched in 2011 with the primary goal of improving access to and facilitating enrolment in clinical trials. The secondary goal was to improve our understanding of the epidemiology of neuromuscular diseases across Canada, as well as to determine regional differences in diagnosis and treatment.

Objectives: The aim of the present study was to perform a descriptive statistical analysis of the amyotrophic lateral sclerosis (ALS) population registered in the CNDR as of April 2014.

Methods: Patients with ALS were recruited into the registry through adult ALS clinics or by self-registration through the CNDR National Office. Data collection at routine visits has been outlined previously (1). For the present study, data from all captured ALS clinic visits for registered ALS patients was accessed. We reviewed data on 358 patients.

Results: The mean age of the study population was 63.5(+/− 12.2) years. The majority of patients were male (61%) and 88% of patients were from urban locations. In our population the site of disease onset was limb-onset in 69% (199/288) of patients, bulbar-onset in 20% (58/288) and the remaining patients had either cognitive onset (0.3%), respiratory (0.6%), mixed onset (8%) or unknown onset (2%). The average percent predicted forced vital capacity (% FVC) at the last clinic visit was 73.8% (standard deviation +/− 25.4). Average ALSFRS-R score at enrolment was 26 (SD +/− 10). We found that 93% of the patients were registered with the Canadian ALS society. Data was available on 311 patients with respect to riluzole use revealing that 209 (67%) were currently taking riluzole whereas 8% reported previous use, 5% declined use and 2% were unknown regarding their riluzole status. There was regional variability with riluzole use, with 21 patients out of 54 (39%) in the Province of Alberta actively using riluzole as compared to 163 patients in the Province of Ontario using riluzole out of 220 (74%). Across the country 47 of 311 patients (15%) were actively involved in a clinical trial, with 15 (5%) having been past clinical trial participants. Only 11% of the ALS patients had personal directives (19 women and 22 men).

Discussion and conclusion: Information regarding 358 patients across Canada was collected and presented in this study in order to provide the first nation-wide epidemiological survey of ALS.

Acknowledgements: This abstract was compiled on behalf of the CNDR Investigator Network.

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P153 PREVALENCE OF AMYOTROPHIC LATERAL SCLEROSIS (ALS) IN THE UNITED STATES, OCTOBER 19, 2010 – DECEMBER 31, 2011

MEHTA P, HORTON K, ANTAO V

ATSDR/CDC, Atlanta, GA, USA

Email address for correspondence: pum4@cdc.gov

Keywords: prevalence, United States, demographics

Background: In October 2010, the federal Agency for Toxic Substances and Disease Registry (ATSDR) launched the congressionally-mandated National ALS Registry to collect and analyze data regarding persons with ALS (PALS) in the United States. ALS, also known as Lou Gehrig's disease, is a progressive neuromuscular disease that usually leads to death within 2–5 years of diagnosis. The initial symptoms of ALS vary and may include muscle weakness in upper or lower extremities along with difficulty speaking, walking, and fatigue and eventual death. The main goals of the Registry are to determine the incidence and prevalence of ALS within the United States, characterize the demographics of those living with ALS, and identify the potential risk factors for the disease.

Objective: To summarize the prevalence of persons with ALS in the United States from October 19, 2010 – December 31, 2011.

Methods: As ALS is not a reportable disease in the United States, the National ALS Registry uses a two-pronged approach to help identify all cases of ALS in the country. The first approach utilizes existing national administrative databases (Medicare, Medicaid, Veterans Health Administration (VHA) and Veterans Benefits Administration (VBA)) to identify prevalent cases. The second method uses a secure web portal to identify cases not included in the national administrative databases. PALS who register via the web portal have the opportunity to complete surveys that may lead to a better understanding of the potential risk factors for ALS (eg, genetics, environmental and occupational influences).

Results: Findings from the National ALS Registry's first report will be presented at the ALS/MND International Symposium in Brussels, December 2014. Descriptive statistics on the prevalence rates of ALS in the US, along with demographics of cases and survey completion rates will be presented.

Conclusion: This report summarizes the prevalence of ALS from PALS from October 19, 2010 – December 31, 2011. This is the first ever effort to identify ALS cases on a national population basis in the United States. The preliminary surveillance results capture ALS prevalence but do not reflect all incident cases, since the ALS diagnosis date was not captured via the national administrative data sets. The establishment of the National ALS Registry will allow for analysis of prevalence of this disease as well as assess potential risk factors that may cause ALS.

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P154 AMYOTROPHIC LATERAL SCLEROSIS (ALS) RE-ADMISSIONS AT CAROLINAS HEALTHCARE SYSTEM - HIGHER THAN STROKE RE-ADMISSIONS

BROOKS BR[1,4], NICHOLS MS[1], LINDBLOM SS[3,4], PACICCO TJ[3,4], BOCKENEK WL[2,4], SANJAK MS[1,5], WARD AL[1,6], LUCAS NM[1], SMITH NP[1], BELCHER SL[1], LARY C[1], NEMETH J[1], RUSSO PC[1], BRAVVER EK[1,4], DESAI UG[1,4], STORY JS[1,4], HOLSTEN SE[1], FISCHER MP[1], LANGFORD VL[1], WRIGHT KA[1]

[1]*Carolinas Neuromuscular/ALS-MDA Center - Carolinas Medical Center - Department of Neurology – Carolinas Healthcare System Neurosciences Institute, Charlotte, North Carolina, USA,* [2]*Department of Physical Medicine and Rehabilitation - Carolinas Rehabilitation, Charlotte, North Carolina, USA,* [3]*Department of Internal Medicine - Carolinas Medical Center, Charlotte, North Carolina, USA,* [4]*University of North Carolina School of Medicine – Charlotte Campus, Charlotte, North Carolina, USA,* [5]*Department of Kinesiology, University of North Carolina - Charlotte, Charlotte, North Carolina, USA,* [6]*Cabbarus College of Health Sciences - Occupational Therapy, Concord, North Carolina, USA*

Email address for correspondence: benjamin.brooks@carolinashealthcare.org

Keywords: hospital admissions, in-patient mortality, medical co-morbidities

Objective: To compare ALS re-admissions prevalence with stroke re-admissions.

Methods: 622 home-visit ALS patients (48% M; 52% F) were monitored prospectively compared with 834 stroke patients.

Results: ALS re-admissions were 17.7% (12.0–24.5%/month) annually greater than Stroke re-admissions-7.4% (1.9–11.9%/month; Chi-Square = 4.4209; p = 0.04). Pneumonias (7%) were more common in ALS re-admissions (Chi-Square = 4.6875; p = 0.03). Re-admission for falls was similar in both ALS (5%) and stroke (2%) patients. Respiratory re-admissions were significantly more common in ALS (28%) than stroke (2%) patients (Chi-Square = 29.4011; $p < 0.01$) as seen previously (1). Cerebrovascular events occurred in 40.3% of the stroke re-admissions while none occurred in ALS re-admissions. Death during hospitalization occurred in 29.3% of ALS re-admissions (5.2% of all ALS home-visit patients) compared with 11.3% of stroke re-admissions (0.8% of all stroke patients; Chi-Square = 12.5; $p < 0.01$).

Conclusion: ALS patients followed in the home care setting constitute a source of significant re-admissions to an acute hospital setting (2). Comparing two cohorts of comparable size identified differences in types of problems leading to re-admission and will provide a framework for planning interventions to decrease these re-admissions.

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P155 BLOOD LEVELS OF TRACE METALS AND AMYOTROPHIC LATERAL SCLEROSIS (ALS) IN US MILITARY VETERANS

PETERS TL[1, 2], BEARD JD[1, 3], UMBACH DM[4], ALLEN K[5, 6], KELLER J[7], MARIOSA D[2], SANDLER DP[1], SCHMIDT S[8], FANG F[2], YE W[2], KAMEL F[1]

[1]*Epidemiology Branch, National Institute of Environmental Health Sciences, NIH, DHHS, Research Triangle Park, NC, USA,* [2]*Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden,* [3]*Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC, USA,* [4]*Biostatistics Branch, National Institute of Environmental Health Sciences, NIH, DHHS, Research Triangle Park, NC, USA,* [5]*Epidemiology Research and Information Center, Durham VA Medical Center, Durham, NC, USA,* [6]*Department of Medicine and Thurston Arthritis Research Center, University of North Carolina, Chapel Hill, NC, USA,* [7]*Westat Inc., Durham, NC, USA,* [8]*Department of Medicine, Duke University Medical Center, Durham, NC, USA*

Email address for correspondence: tracy.peters@ki.se

Keywords: metals, case control, epidemiology

Background: Past studies have reported higher concentrations of trace metals such as selenium (Se), zinc (Zn), copper (Cu) and manganese (Mn) in ALS cases compared to controls. Deficiencies of trace metals might also affect ALS risk, but only Zn deficiency has been studied.

Objectives: To examine associations of trace metals (Se, Zn, Cu and Mn) with ALS risk and to evaluate whether these associations differed by site of disease onset (bulbar or spinal).

Methods: We conducted a case-control study of 139 medical record-confirmed cases recruited in 2007 from the US Department of Veterans Affairs (VA) National Registry of Veterans with ALS and 229 veteran controls frequency matched to cases on age and use of VA medical care. Whole blood samples were collected in trace metal-free tubes; metals were measured using inductively coupled plasma mass spectrometry. Associations between metals and ALS risk were evaluated using unconditional logistic regression to estimate odds ratios (ORs) and 95% confidence intervals (CIs) after adjustment for age, gender, and race/ethnicity.

Results: Blood metal levels in controls were comparable to those observed in other populations. Associations of ALS risk with Se and Zn were inverse; comparing the highest to the lowest 20%, the OR (95% CI) for Se was 0.4 (0.2–0.8, $P_{trend} = 0.02$) and that for Zn was 0.6 (0.3–1.2, $P_{trend} = 0.03$). Cu was associated with increased ALS risk (OR 2.5, 1.2–4.9, $P_{trend} = 0.01$) as was Mn, but the strongest association for the latter was with the middle 20% (OR 2.3, 1.2–4.6) and there was no linear trend ($P_{trend} = 0.79$). Additional adjustment for smoking did not change associations. Adjustment for lead attenuated the positive association with Cu (OR 1.9, 0.9–3.9, $P_{trend} = 0.13$) but did not change associations with other metals. Both positive and inverse associations were stronger in individuals with bulbar compared to spinal onset.

Discussion and conclusion: Our study shows that higher levels of Zn and Se within the normal range are associated with decreased ALS risk after adjusting for other known risk factors. In contrast, higher levels of Cu are associated with increased ALS risk, consistent with past literature, but this relationship may be explained partly by lead. Most previous literature has focused on increased ALS risk associated with higher levels of Se and Zn. However, although we cannot exclude reverse causality, it is possible that deficiencies of these metals are also associated with ALS risk.

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P156 SOD1 GENE MUTATIONS IN ALS PATIENTS TURKISH POPULATION

IDRISOGLU HA, POLAT N, IDRISOGLU M

Medical Faculty, Istanbul, Turkey

Email address for correspondence: haidris@istanbul.edu.tr

Keywords: Turkish population, SOD1

Background: Amyotrophic lateral sclerosis (ALS) is a fatal late onset neurological disorder characterized by motor neuron degeneration in the primary motor cortex, brainstem and spinal cord. The majority of cases are sporadic and only 5–10% have a family history. Familial ALS cases show a high heritability and this has enabled the identification of several genetic triggers, of which mutations in SOD1, FUS, TARDBP and C9ORF72 are the most frequent.

Methods: We have searched for the SOD1 gene mutation in 770 ALS patients. 700 of 770 patients were sporadic and the others were familial ALS (30 families).

Results: The SOD1 gene mutation was not detected in any family. The SOD1 gene mutation was detected in 0.5% of patients who came to the clinic with sporadic ALS. The family research of 2 patients with SOD1 gene mutation was present without the clinical and electrophysiological findings in the family members. SOD1 gene mutations were negative in our laboratory, but were positive in another laboratory in one ALS patient.

Conclusions: We observed that in both sporadic and familial ALS patients the D90A SOD1 mutation was not causative for ALS pathogenesis in the Turkish population.

The SOD1 gene mutation was found in 0.7% of 700 patients in both familial ALS and sporadic ALS cases. We also observed good progression in the patient with SOD1 gene mutation. There was no SOD1 mutation in any of the patients with familial ALS.

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P157 NATURAL HISTORY AND CLINICAL FEATURES OF SPORADIC AMYOTROPHIC LATERAL SCLEROSIS IN CHINA: A TEN-YEAR CLINIC-BASED COHORT STUDY

CHEN L, ZHANG B, TANG L, YE S, LIU X, MA Y, ZHANG H, FAN D

Peking University Third Hospital, Beijing, China

Email address for correspondence: dsfan2010@aliyun.com

Keywords: natural history, clinical features, prognostic predictors

Background: ALS is a fatal neurodegenerative disorder. The characteristics of Chinese ALS patients have not been studied yet. In this clinic-based cohort study, we aimed to provide data on natural history, clinical features and prognostic predictors of ALS in China.

Methods: Between January, 2003 and December, 2012, all patients with a diagnosis of ALS referred to and assessed at PUTH were screened with a follow-up every three months by telephone. Baseline demographic details and clinical data were collected from the patient's first visit to PUTH and follow-up visits. Survival and tracheotomy were predefined primary outcome measures. Group differences were analysed by parametric tests and non-parametric tests as appropriate. Survival was calculated using a Kaplan-Meier analysis, survival rate was calculated using a life table analysis and predictors were identified in a Cox regression model.

Results: In the 1624 recruited cases, 1220 (75.1%) were limb-onset ALS, 227 (14.0%) bulbar-onset ALS, 126 (7.8%) FAS, 43 (2.6%) PMA and others (8 cases, 0.5%) . The overall M: F ratio was 1.7:1 and the mean age of symptom onset was 49.8 years. The overall median diagnostic delay time from symptom onset was 14.0 months (Range, 0–228). Median survival from symptom onset as determined by Kaplan-Meier analysis was 71 months. The mean onset age, sex ratio, BMI, diagnostic delay time and the median survival time were different between phenotypes. The percentage of smokers was different between phenotypes ($p = 0.013$). The mean onset age, the percentage of each phenotype and the median survival time were different between male and female. There were more male patients who had the history of long-time smoking, alcohol abuse, drinking tea frequently or always eating aginomoto or had contact history of harmful gas than female patients. Survival of patients was associated with sex, age of symptom onset, BMI, diagnostic delay time, phenotype of the disease, Airlie House category at presentation, residence, use of traditional Chinese medicine and contact history of pesticides.

Conclusion: Our prospective cohort study provides important information of Chinese ALS patients based on the data from mainland China for the first time, which will be helpful for neurologists in patient counselling and designing of future clinical research studies.

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THEME 7 GENETICS

P158 COMPLETE MUTATIONAL SPECTRUM OF KNOWN ALS GENES IN A LARGE COHORT OF FAMILIAL ALS CASES

MÜLLER K[1], MARROQUIN N[1], VOLK AE[1,5], HÜBERS A[1], MEYER T[3], ANDERSEN PM[1,4], MEITINGER T[2], LUDOLPH AC[1], STROM TM[2], WEISHAUPT JH[1]

[1]*Ulm University, Ulm, Germany,* [2]*Helmholtz Institute, Munich, Germany,* [3]*Humboldt University Berlin, Berlin, Germany,* [4]*Umea University, Umea, Sweden,* [5]*University Medical Center Hamburg-Eppendorf, Hamburg, Germany*

Email address for correspondence: kathrin.mueller@uni-ulm.de

Keywords: next generation sequencing, common ALS genes, familial ALS

Recent advances in ALS genetics have led to the discovery of approximately 25 different genes mutated in ALS with a mostly autosomal-dominant pattern of inheritance. However, the complete spectrum of mutations in all known ALS genes, and their contribution to ALS has never been determined in a homogenous large cohort of familial ALS cases.

We thus combined Sanger sequencing, fragment length analysis, repeat-primed PCR, Southern blotting and whole exome sequencing to obtain a comprehensive profile of ALS gene mutations in more than 150 German ALS families.

We report the relative contribution of each ALS gene to familial ALS and surprisingly show a higher proportion of FUS cases (4%) compared to TARDBP (2.5%) in Germany. Moreover, we identified several novel mutations, and demonstrate absence of mutations in some recently described ALS genes. 55% of German familial ALS cases did not carry a mutation in any of the known ALS genes. 81% of the genetically defined cases showed mutations in the four most common ALS genes C9ORF72, SOD1, FUS and TARDBP, while the individual relative contribution of the other ALS genes in our cohort was very low (19%).

However, in these rare ALS genes several seemingly unique mutations that have not been described in other families were observed. Our data support the assumption that, beyond the 4 most frequent genes, ALS is a genetically highly heterogeneous disease.

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P159 A DUTCH FAMILY WITH AUTOSOMAL RECESSIVE MOTOR NEURON DISEASE CAUSED BY OPTINEURIN MUTATIONS

BEELDMAN E[1], VAN RUISSEN F[2], BAAS F[2], DE VISSER M[1], VAN DER KOOI A[1]

[1]*Department of Neurology,* [2]*Department of Genome Analysis, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands*

Email address for correspondence: e.beeldman@amc.uva.nl

Keywords: optineurin (OPTN), case report, familial ALS

Background: Pathogenic mutations in the optineurin gene (OPTN) are infrequently found in patients with motor neuron disease (MND). We report the first Dutch family with autosomal recessive MND caused by mutations in the OPTN gene.

Case reports: A 21-year old healthy woman (2.2) presented with a 4-month history of weakness of her left leg and a bilateral foot drop. Neurological examination showed fasciculations, mild to moderate weakness of the upper and lower extremities, respectively, with symmetrical tendon reflexes and indifferent plantar responses. Electromyography (EMG) showed denervation in the lower extremities with signs of reinnervation in the right leg. Her symptoms progressed rapidly and she received a tracheostomy early in the disease course. She died 15 years after symptom onset. Her sister, a 27-year old healthy woman (2.1) and 4 months pregnant, presented with a 4-month history of fasciculations and weakness of the left arm. Neurological examination revealed a Trendelenburg's sign on the left and atrophy with severe weakness of the proximal muscles of the left arm and mild weakness of the right arm and leg. Tendon reflexes were reduced on the left with an indifferent plantar response. She fulfilled the El Escorial criteria in the cervical and lumbosacral regions. She died 12 months after symptom onset due to acute respiratory failure. Their brother (2.3) presented to our hospital at age 38, with a one-year history of fasciculations. Neurological examination showed fasciculations of his back and extremities with mild proximal weakness of both arms. Tendon reflexes were symmetrical with normal plantar responses. On EMG, he fulfilled the El Escorial criteria in the cervical, thoracic and lumbosacral regions. Eighteen months after symptom onset he received non-invasive ventilation and a feeding tube. After thirty months he was completely bed-bound and slightly dysarthric. He lives at a nursing home and spends the weekends at home with his wife and kids. Genetic testing of patients 2.1 and 2.3 showed two mutations in the OPTN gene, a c.658delG and c.493C>T mutation. The c.658delG mutation was found in their father and the c.493C>T mutation in their mother. Both parents are alive and healthy. Genetic analysis of patient 2.2 was not available.

Discussion and conclusion: To the best of our knowledge this is the first Dutch family with autosomal recessive MND based on two mutations in the OPTN gene. The c.493C>T mutation has been described in a Danish MND family with a possible autosomal dominant inheritance. However, both mutations result in a stop codon, which most likely leads to an optineurin deficiency in our patients. It is therefore unlikely that a heterozygous c.493C>T mutation is pathogenic. Based on the clinical presentation and the family history, an autosomal recessive inheritance is highly probable in our patients.

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P160 SPECTRUM OF MUTATIONS IN ALS GENES ON THE ISLAND OF SARDINIA

CHIÒ A[1], FLORIS G[2], MARROSU F[2], MARROSU MG[2], MURRU MR[2], PUGLIATTI M[3], PARISH LD[3], CALVO A[1], MOGLIA C[1], TRAYNOR BJ[5], RENTON AE[5], BRUNETTI M[1], OSSOLA I[1], BARBERIS M[1], RESTAGNO G[3], BORGHERO G[2]

[1]*University of Turin, Turin, Italy,* [2]*University of Cagliari, Cagliari, Italy,* [3]*AOU Città della Salute e della Scienza, Torino, Italy,* [4]*University of Sassari, Sassari, Italy,* [5]*NIH, Bethesda, USA*

Email address for correspondence: achio@usa.net

Keywords: gene, Sardinia, epidemiology

Background: Sardinia, the second largest Mediterranean island, represents a genetic isolate; its population displays decreased genetic and allelic heterogeneity. We have already reported that ALS patients of Sardinian ancestry have frequency higher than expected of the *TARDBP* p.A382T missense mutation.

Aims: We report the genetics of a larger series of ALS patients of Sardinian ancestry extending our analysis to include other ALS genes and their clinical correlates.

Methods: All ALS patients of Sardinian ancestry were eligible to be included in the study. Patients were identified through the ITALSGEN consortium in the period 2008 to 2013. *SOD1, TARDBP, FUS, OPTN* and *ANG* genes were PCR amplified, sequenced using the Big-Dye Terminator v3.1 sequencing kit (Applied Biosystems Inc.), and run on an ABI-Prism 3130 genetic analyzer. A repeat-primed PCR assay was used to screen for the presence of the GGGGCC hexanucleotide expansion in the first intron of *C9ORF72*. A cut-off of ≥ 30 repeats combined with a typical sawtooth pattern was considered pathological.

Results: Out of a total of 375 ALS cases of Sardinian ancestry, 155 (41.3%) carried mutations in one or more genes. Of these: 75 patients (20.0%) carried a *TARDBP* heterozygous p.A382T missense mutation; 3 (0.8%) a *TARDBP* homozygous p.A382T mutation; 10 (2.7%) a *TARDBP* heterozygous p.G295S mutation; one a *TARDBP* homozygous p.G295S mutation; and one a double heterozygous mutation (p.G295S and p.A382T) of *TARDBP* gene. 51 patients (13.6%) carried a hexanucleotide repeat expansion of the *C9ORF72* gene and 8 (2.1%) a double mutation of *TARDBP* (p.A382T) and of *C9ORF72*. Four patients (1.1%) had a missense mutation of the *SOD1* gene (p.A95G and p.A4T) and two a p.T622A missense mutation of the *MATR3* gene.

A *TARDBP* p.A382T heterozygous missense mutation has been detected in eight of the 700 control samples (1.1%); the relative risk of developing ALS in a subject carrying this mutation was 66.2 (95% CI, 32.9-141.9).

Subjects carrying the *C9ORF72* repeat expansion had a higher frequency of bulbar onset. The age at symptom onset differed between genetic subgroups; in particular, patients with co-occurrence of *C9ORF72* and the *TARDBP* p.A382T missense mutation had ~20 years lower age at onset. Frontotemporal dementia, identified in 51 patients (13.6%), was more frequent in patients with *C9ORF72* mutations.

Discussion and conclusion: In Sardinian ALS patients genetic mutations accounted for 75% of FALS and 30% of apparently SALS, representing the largest proportion of ALS that is genetically explained in a population outside of Finland. The most common mutations were the p.A382T and p.G295S missense mutations in *TARDBP* and the pathogenic repeat expansion of *C9ORF72.* Several patients carried a double mutation, more commonly the combination of *C9ORF72* and *TARDBP* p.A382T missense mutation, and a smaller number were homozygous for *TARDBP* missense mutations.

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P161 TARDBP MUTATION MIMICS A DISTAL MOTOR NEUROPATHY IN A SARDINIAN PATIENT

CALDARAZZO EI[1], CARLESI C[1], LO GERFO A[1], CHICO L[1], MANCUSO M[1], FOGLI A[2], SIMI P[2], SICILIANO G[1]

[1]*Department of Clinical and Experimental Medicine, Neurological Clinic, University of Pisa, Italy,* [2]*Department of Medical Laboratories, Medical Genetics Laboratory, University Hospital of Pisa, Italy*

Email address for correspondence: elenacaldarazzoienco@gmail.com

Keywords: TARDBP, p.A382T mutation, Sardinia

Background: TARDBP-related Amyotrophic Lateral Sclerosis (ALS) patients present an adult-onset, autosomal dominant clinically typical form of ALS. TARDBP mutations are also observed in both ALS-Fronto Temporal Dementia (FTD) and pure FTD cases. Since first TARDBP mutations were reported in familial ALS cases in 2008, over 40 mutations have been identified in several populations of different geographic origin.

Objectives: Here we report an atypical case of TARDBP-associated ALS patient, coming from Sardinia, an Italian island historically genetically segregated and distinct from other European populations.

Care report: A 50-yr old man came to our attention for a 10-year story of slowly progressive mild symmetrical limb distal hyposthenia and amyotrophy with cramps and fasciculation. No upper motor neuron sign or sensitive impairment was present. Electrophysiological examinations were consistent with second motor neurons damage. A psychiatric history of bipolar disorder was present without cognitive impairment. No family history of neuromuscular disorders.

Results: Genetic analysis revealed that the patient was carrying in heterozygosis the c.1144G->A (p.A382T) pathogenic missense mutation of the TARDBP gene.

Discussion and conclusion: TARDBP p.A382T missense mutation accounts for approximately one-third of all ALS Sardinian cases. Despite a quite heterogeneous spectrum of resulting phenotypes, the flail arm variant of ALS occured with greater than expected frequency in these patients, although clinical presentation may also include forms of parkinsonism and FTD. To our knowledge, this is the first report of a distal motor neuropathies-like syndrome associated with this mutation.

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P162 ARHGEF28 GENE EXON 6/INTRON 6 JUNCTION MUTATIONS IN CHINESE AMYOTROPHIC LATERAL SCLEROSIS COHORT

MA Y, TANG L, CHEN L, ZHANG B, FAN D

Peking University Third Hospital, Beijing, China

Email address for correspondence: dsfan2010@aliyun.com

Keywords: ARHGEF28 gene, RGNEF protein, ALS cohort

Objective: To analyze the intron 6, +1 delG (GT>TT) mutation in Chinese patients with amyotrophic lateral sclerosis (ALS), investigate the incidence of this mutation, and identify the relationship between the genotype and phenotype in Chinese ALS patients.

Methods: We sequenced this mutation in 25 familial ALS (fALS) cases, 357 sporadic ALS (sALS) patients, and 442 healthy control subjects. We collected blood samples and screened for the intron 6, +1 delG (GT>TT) mutation by extraction of genomic DNA, PCR, direct sequencing, and TA cloning.

Results: Two sALS patients exhibited the intron 6, +1 delG (GT>TT) mutation of the ARHGEF28 gene. Thus, the incidence of the mutation was 0.52% (2 cases/382 cases) in all of the ALS patients and 0.56% (2 cases/357 cases) in the sALS subgroup. The clinical features of the mutation-positive patients were quite different from those reported in the literature. These characteristics differed in terms of sex, site of onset, cognitive function, and family history.

Conclusion: The intron 6, +1 delG (GT>TT) mutation of the ARHGEF28 gene is present in the Chinese population; however, the resultant phenotype differs from those observed in other ethnic groups.

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P163 FIVE NOVEL SQSTM1 MUTATIONS IN A CHINESE AMYOTROPHIC LATERAL SCLEROSIS COHORT

YANG Y, TANG L, ZHANG N, FAN D

Peking University Third Hospital, Beijing, China

Email address for correspondence: dsfan2010@aliyun.com

Keywords: p62 protein, SQSTM1, novel mutation

Objective: The SQSTM1 gene encodes the p62 protein. Mutations in this gene have been previously reported in patients with familial and sporadic amyotrophic lateral sclerosis (ALS). The purpose of this study was to identify mutations in the SQSTM1 gene and to determine the survival time based on the progression rate of the ALSFRS-R score.

Methods: We sequenced the SQSTM1 gene in 471 Chinese patients with sporadic and familial ALS from 2011 to 2013. SQSTM1 gene mutations were screened using PCR and direct sequencing, and genotype-phenotype correlations and the progressive ALSFRS-R ratio were analyzed.

Results: Seven heterozygous missense mutations were detected in 471 ALS patients. We identified five novel missense mutations: c. 241 G>A p. E81K in the PB1 domain, c. 717 C>A p. N239K in the TRAF6 domain, c.889 G>A p. G297S and c. 1116 G>C p. E372D in the PEST2 domain, and c. 1162 C>T p. P388S in the UBA domain.

Conclusion: The SQSTM1 mutations present in Chinese patients suggest that this gene is involved in ALS cases worldwide. Most of the clinical phenotypes of this mutation varied greatly among different patients.

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P164 EVIDENCE OF COMMON GENETIC VARIATION FOR ALS RISK IN CHINESE SAMPLES

BENYAMIN B[1], HE J[2,1], LEO P[1], SHAH S[1], HEMANI G[3], CREMIN K[1], MANGELSDORF M[1], WRAY N[1], XU H[4], BARTLETT PF[1], BROWN M[1], VISSCHER P[1], FAN D[2]

[1]*The University of Queensland, Brisbane, Australia,* [2]*Peking University Third Hospital, Beijing, China,* [3]*The University of Bristol, Bristol, UK,* [4]*Second Military Medical University, Shanghai, China*

Email address for correspondence: b.benyamin@uq.edu.au

Keywords: GWAS, methylation, gene

Background: Genetic factors are a major cause of ALS even in apparently sporadic cases. Currently, the known ALS genes explain a small proportion of sporadic cases. Except for age and sex, there are no specific biomarkers and environmental factors known that affect ALS. Thus, elucidating the genetic aetiology of ALS is a key to its treatment and cure.

Objectives: We aim to discover novel genes affecting ALS using a genome-wide association study (GWAS) in a Chinese ALS case-control cohort.

Methods: We used genome-wide single nucleotide polymorphisms (SNPs) data from Illumina OmniZhongHua-8 V1 genotyping arrays from 1,324 cases and 3,115 controls. After quality control, we performed a number of analyses in a cleaned dataset of 1,243 cases and 2,854 controls. These include: (i) a genome-wide association analysis to identify SNPs associated with ALS using PLINK software; (ii) GREML analysis to estimate the proportion of the phenotypic variance in ALS liability due to common SNPs; (iii) gene-based analysis to identify genes associated with ALS. Genome-wide methylation data from the Illumina 450K array was also available in a subset of samples (501 cases and 198 controls).

Results: There were no genome-wide significant SNPs and genes associated with ALS. However, we estimated that 17% (SE: 0.05; $p = 6 \times 10^{-5}$) of the phenotypic variance in ALS liability was due to common SNPs. The top associated SNP was within GNAS (Guanine Nucleotide Binding Protein (G Protein), Alpha Stimulating Activity, rs4812037; $p = 7 \times 10^{-7}$). GNAS was also the most associated gene from gene-based study ($p = 2 \times 10^{-5}$). The analysis of methylation data showed that rs4812037 was differently methylated in 2 out 173 *GNAS* probes (cg10797197; $P = 1 \times 10^{-16}$ and cg17696847; $p = 2 \times 10^{-8}$).

Discussion and conclusion: The failure to identify a genome-wide (GW) significant variant is likely due to the limited power of this study to find variants with small effects. However, a significant proportion (17%) of ALS liability that can be explained by common SNPs indicated that with an increase in sample size, GW significant variants can be identified. To achieve that, we are currently performing a meta-analysis of our Chinese GWAS results with the largest

European ALS GWAS of 6100 cases and 7100 controls (the results are pending). The significant associations between the top SNP and the two *GNAS* probes also showed that *GNAS* is an interesting candidate, and methylation data can provide biological support for the identified genetic variant-disease associations.

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P165 C9ORF72 HEXANUCLEOTIDE REPEAT EXPANSIONS ARE RARE AND HYPER-METHYLATED IN CHINESE SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

HE J[1,2], TANG L[1], BENYAMIN B[2], SHAH S[2], HEMANI G[2], LIU R[1], YE S[1], LIU X[1], MA Y[1], ZHANG H[1], CREMIN K[3], LEO P[3], WRAY N[2], VISSCHER P[2], XU H[4], BROWN M[3], BARTLETT PF[2], MANGELSDORF M[2], FAN D[1]

[1]*Peking University Third Hospital, Beijing, China,* [2]*Queensland Brain Institute, University of Queensland, Brisbane, Australia,* [3]*University of Queensland Diamantina Institute, Brisbane, Australia,* [4]*Shanghai Changzheng Hospital, Second Military Medical University, Shanghai, China*

Email address for correspondence: dsfan2010@aliyun.com

Keywords: C9ORF72, haplotype, methylation

Background: A hexanucleotide repeat expansion (HRE) in the *C9ORF72* gene has been identified as the most common mutation in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) patients among Western populations.

Methods: We performed fragment-length and repeat-primed PCR to determine GGGGCC copy number and expansion within the *C9ORF72* gene in 1,092 sporadic ALS (sALS) and 1,062 controls from China. We performed haplotype analysis of 23 SNPs within and surrounding the *C9ORF72* gene. Lastly, CpG methylation was assessed in the region of *C9ORF72* gene.

Results: The *C9ORF72* HRE was found in three sALS patients (0.3%) but not in control subjects ($p = 0.25$, Fisher's exact test). Two cases with the HRE did not harbor four risk alleles that have previously been determined to be strongly associated with ALS in Caucasian populations. Several risk alleles (including rs2814707 and rs384992) of the 20-SNP consensus risk founder haplotype in Caucasians demonstrated that two of the three cases shared a novel haplotype carrying the repeat expansion. Two of the three HRE carriers showed hyper-methylation of the CpG island upstream of the repeat that was not detected in other sALS patients ($p < 10^{-8}$) or controls.

Discussion and conclusion: The low frequency (1.8%) of the 20-SNP consensus risk haplotype and the distinct allele distribution in Chinese sALS patients compared to Caucasian populations indicates that the *C9ORF72* HRE is not from the same single founder haplotype involved in Caucasian populations. The extreme methylation pattern of the CpG island upstream of the repeat in two of the HRE carriers is consistent with the repeat expansions being causal ALS mutations.

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P166 ANALYSIS OF C9ORF72 REPEAT EXPANSION IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS FROM SOUTHWEST OF CHINA

SHANG H, CHEN Y, CHEN X

West China Hospital, Sichuan University, Chengdu, Sichuan, China

Email address for correspondence: hfshang2002@126.com

Keywords: C9ORF72, repeat expansion, Southwest of China

An intronic GGGGCC hexanucleotide repeat expansion in the C9ORF72 gene was identified as the most common cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia in Western populations. Using the repeat-primed polymerase chain reaction analysis, we screened for C9ORF72 in a cohort of sporadic ALS patients of Chinese origin ($n = 640$).

No pathogenic repeats (>30 repeats) were detected in either the patients or control subjects, indicating that the pathogenic expansions of C9ORF72 might be a rare cause of ALS in Southwest of China.

To the best of our knowledge, this is the first and largest study to investigate the correlation between C9ORF72 and ALS patients from Southwest of China. Additionally, the results of this study suggest that it would seem pointless to screen for this pathogenic expansion in Chinese patients with this fatal neurodegenerative disease.

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P167 A BLINDED COMPARATIVE STUDY ON THE RELIABILITY OF GENETIC TESTING FOR THE GGGGCC-REPEAT EXPANSION IN C9ORF72 PERFORMED IN 14 LABORATORIES

AKIMOTO C[1], VOLKAE[2], NORDIN A[1], ANDERSEN PM[1], KUBISCH C[2]

[1]*Department of Pharmacology and Clinical Neuroscience, Umeå University, Umeå, Sweden,* [2]*Institute of Human Genetics, Ulm University, Ulm, Germany*

Email address for correspondence: angelica.nordin@neuro.umu.se

Keywords: genetic testing, C9ORF72, RP-PCR

Background: The GGGGCC-repeat expansion in C9ORF72 is the most frequent mutation found in patients with amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Most of the studies on C9ORF72 have relied on repeat-primed PCR (RP-PCR) for detection of an expansion, a cheap and fast method but it cannot provide size measurements for large expansions and may be inaccurate.

Objectives: To analyse the reliability of the RP-PCR technique for genotyping the GGGGCC-repeat expansion in C9ORF72.

Method: To investigate the inherent limitations of RP-PCR, we compared methods and results of 14 laboratories, which genotyped DNA from 78 ALS and FTD patients in a blinded fashion. Eleven laboratories used a combination of amplicon-length analysis and RP-PCR, whereas three laboratories used RP-PCR alone. Southern blot was performed in three laboratories as a reference.

Results: The mean sensitivity of RP-PCR alone was 94.3% (71.7-100%) and the mean specificity was 97.3% (87.5-100%). Combining the results of RP-PCR and amplicon-length analysis, 100% sensitivity and specificity were found in only five laboratories (A-E, 35.7%), whereas sensitivity and specificity above 95% were found in seven laboratories (A-E, G and L, 50%). Using PCR-based techniques, only 5 of the 14 laboratories got results in full accordance with Southern blot analysis.

Conclusion: There was a high degree of false positive and false negative results. We recommend using a combination of amplicon-length analysis and RP-PCR as a minimum in a research setting. Southern blot should be the gold standard and obligatory in a clinical diagnostic setting.

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P168 VARIATION IN SIZE OF THE C9ORF72 GGGGCC-REPEAT EXPANSION BETWEEN DIFFERENT TISSUES IN ALS AND FTD

NORDIN A[1], AKIMOTO C[1], FORSBERG K[2], GRAFFMO K[2], ALSTERMARK H[1], BRÄNNSTRÖM T[2], ANDERSEN P[1]

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

Email address for correspondence: angelica.nordin@neuro.umu.se

Keywords: C9ORF72, repeat expansion, southern blot

Introduction: Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are fatal neurodegenerative disorders genetically linked to one another. The most common genetic cause for both conditions is a large GGGGCC-repeat expansion in C9ORF72 (1–2). Repetitive sequences are often unstable which potentially can lead to changes in number of repeats between generations and during somatic cell division. Changes arising during somatic cell mitosis will result in populations of cells with varying expansion patterns, eg, one expansion size in neurons and another size in leucocytes. This could potentially give rise to problems regarding diagnosis, but might also explain some of the observed phenotypic differences among carriers of C9orf72-expansions.

Objectives: The aims of this study were 1) to determine whether the size of the GGGGCC-repeat expansion in C9ORF72 differs between different tissues in patients with ALS, FTD or ALS+FTD and 2) to determine whether there are any correlations between repeat number and phenotype.

Methods: Using Southern blot we have analyzed a broad panel of tissues, consisting of autopsy material of both neuronal and non-neuronal origin with a focus on brain, spinal cord and muscle, from 17 individuals with ALS, PBP, FTD or ALS+FTD, all with an expanded allele in blood. The number of repeats in each sample was determined and compared between different tissues within individuals. The expansion size pattern between individuals with different diagnoses was also compared.

Results: We see marked differences in expansion sizes between different tissues within patients, in some cases 1000–2000 repeats difference between tissues. No correlations between expansion size and disease phenotype were apparent. In line with previous findings (3), we have found the expansion sizes in the cerebellum to be smaller than in other neuronal tissues and also observed patients which have a very small expansion size in blood, muscle and internal organs (approx. 60 repeats), but massive expansions in brain tissue.

Conclusions: There are differences in size of the C9ORF72 GGGGCC-repeat expansion between tissues of different origin. However, the expansion size pattern seems to be rather individual and not correlated to a specific disease phenotype.

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P169 SCREENING FOR C9ORF72 REPEAT EXPANSION IN AMYOTROPHIC LATERAL SCLEROSIS

MOSCA L[1], TARLARINI C[1], LUNETTA C[2], SANSONE V[2], PENCO S[1]

[1]*Department of Laboratory Medicine and Microbiology, Medical Genetics,* [2]*NEuroMuscular Omnicentre, Fondazione Serena Onlus, Niguarda Ca' Granda Hospital, Milano, Italy*

Email address for correspondence: silvana.penco@ospedaleniguarda.it

Keywords: C9ORF72, genetics, molecular tests

Background: ALS is familial in 10–15% of cases (FALS), while for the majority of cases it is sporadic (SALS) (1). To date more than 17 causative genes have been described; the most commonly mutated are: *SOD1*, *FUS*, *TARDBP* and *C9ORF72*. The *C9ORF72* mutation is a polymorphic hexanucleotide (GGGGCC) repeat expansion located in intron 1 (3); was and has been shown to be the most common genetic cause of FALS, FTD and ALS-FTD. The phenotype associated with the pathological expansion is extremely variable and, with few exceptions, the variant does not appear to be fully penetrant; this raises the possibility that the expansion may be a risk factor for disease and may not be capable of producing disease in isolation (4).

Objectives: Determination of the prevalence of *C9ORF72* repeat expansion in our cohort of 753 Italian ALS patients.

Methods: *C9ORF72* expansion was analyzed by using the repeat primed PCR (RP-PCR) (3) to detect repeat numbers of approximately maximum 60. The method is able to discriminate the repeat range detected in the normal population (0–20) from the higher mutated range (>30). Genomic DNA extraction and PCR set-up have been performed on an automated Beckman Coulter Biomek NX[P] Workstation. Analysis was performed on an ABI 3730 DNA Analyzer (Applied Biosystems) and GeneMapper software (version 4.0, ABI)

Positive subjects were defined in presence of a repeat number > 30 and/or the typical saw-tooth pattern with a 6-bp periodicity in RP-PCR.

Results: Our ALS cohort consisted in 51 FALS cases (6.8%) and 702 SALS patients (93.2%) (n = 753). FTD was present in 2 FALS (3.9%) and 16 SALS patients (2.3%). The *C9ORF72* pathological expansion was identified in 38 ALS patients (5%). Interestingly, 2 of the *C9ORF72* expansion carriers also presented a causative mutation in one of other ALS-associated genes (*TARDBP* and *FUS*, respectively).

Discussion and conclusion: *C9ORF72* hexanucleotide repeat expansions have been found in 5% of all ALS cases and represent the commonest mutation in our population of Italian ancestry; these data are quite in agreement with previous reports (5) (5% vs 6.7%) obtained in a prospectively ascertained, population-based epidemiologic series of cases identified through the Piemonte and Valle d'Aosta register for ALS (PARALS). Further efforts are needed to implement, in terms of intra- and inter-laboratory reproducibility, the method used for *C9ORF72* analysis.

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P170 IDENTIFICATION OF NEW GENETIC DETERMINANTS IN SPORADIC ALS

COUTHOUIS J, RAPHAEL A, GITLER A

Stanford University, Stanford, CA, USA

Email address for correspondence: julienc@stanford.edu

Keywords: targeted sequencing, genetic determinants, yeast

Background: Most of the studies to identify causative genes in ALS have been carried out in familial ALS (fALS) and identified genes were then often confirmed to be mutated in sporadic ALS (sALS) cases as well (1, 2). With very few large multi-generational ALS pedigrees available, new approaches are needed to expand the genetic landscape of ALS beyond fALS studies.

We previously performed a yeast screen of human RNA Recognition Motifs (RRMs) containing genes looking for candidates mimicking the behaviour of well-known ALS disease genes, *TARDBP* and *FUS* (3, 4). We also previously used trios - families with an affected proband and two unaffected parents - to uncover *de novo* mutations that are only present in the affected proband (5). These approaches allowed us to identify new ALS disease genes *TAF15*, *EWSR1* and *SS18L1*/*CREST* and 62 other potential candidates.

Objectives: Here we wanted to take advantage of new high-throughput sequencing methods to study sporadic samples available to us to identify new ALS disease genes or variants.

Methods: We undertook a targeted sequencing approach of all exons in 169 known and candidate ALS disease genes in 242 sporadic ALS cases and 129 age-matched controls to try to identify novel variants linked to ALS.

Results: We sequenced all exons of 169 genes in 242 ALS patients and 129 age-matched controls from North America. We observed an overall enrichment in novel and rare variants in cases versus controls. Additionally, we identified new variants in known ALS disease genes, in genes associated with ALS, in genes identified through our previous RNA Recognition Motifs (3,4) and trios - families with an affected proband and two unaffected parents - studies (5). While no single gene emerged as significantly enriched in our analysis, we did find several genes that trended towards more novel and rare variants in ALS patient samples versus controls.

Conclusion: We took advantage of affordable recently developed target sequencing methods to rapidly sequence a set of candidate genes in patients versus controls to ask if we could (1) identify new mutations in known ALS disease genes, (2) find further evidence that genes previously associated with ALS are indeed causative, (3) find new mutations in candidates generated from our previously performed study.

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P171 INVESTIGATING THE GENETIC BASIS OF AMYOTROPHIC LATERAL SCLEROSIS USING NEXT-GENERATION SEQUENCING TECHNIQUES

FIFITA J[1], WILLIAMS K[1], NICHOLSON G[1,2], ROWE D[1], BLAIR I[1]

[1]*Australian School of Advanced Medicine, Macquarie University, Sydney, NSW, Australia,* [2]*Molecular Medicine Laboratory, Concord Hospital, Syndey, NSW, Australia*

Email address for correspondence: jennifer.fifita@students.mq.edu.au

Keywords: genetics, novel gene mutation, exome sequencing

Background: The majority of amyotrophic lateral sclerosis (ALS) cases occur sporadically, however 10% of cases show familial inheritance. ALS is genetically heterogeneous with mutations identified in over 15 genes. To date, known gene mutations account for about 60% of familial cases, and for about 5% of sporadic cases in Australian patient cohorts.

Objectives: We aim to identify novel gene mutations that cause familial ALS using exome sequencing, NGS bioinformatic pipelines and computer scripts, and high-throughput screening of control cohorts. Candidate sequence variants will be prioritised for functional studies using computational biol-

ogy. Functional studies will use neuronal cell lines and zebrafish to examine the consequence of each candidate sequence variant.

Methods: A cohort of Australian ALS families (n = 201) has been recruited. An index patient from each family was screened for known ALS genes using both Sanger and exome sequencing. Three families negative for known ALS mutations were selected for gene discovery. Exome sequencing was used to search for novel ALS-linked sequence variants in these families. A custom bioinformatics pipeline was used to filter exome sequence variants and generate a list of candidate variants for each family. Variants were validated with Sanger sequencing. To prioritise variants, their functional effects will be predicted using programs such as MutationTaster, Polyphen, Pon-P2. Protein-protein interactions will be queried using DAPPLE and BioGRID.

Results: Analysis of known ALS genes has identified causative mutations in 62% of Australian familial ALS cases in our cohort (*C9ORF72* 39.3%, *SOD1* 15.4%, *FUS* 2.5%, *TARDBP* 2.0%, *UBQLN2* 1.0%, *OPTN* 0.5%, *SS18L1* 0.5%). Ten DNA samples from the three selected ALS families underwent exome capture and parallel sequencing. This identified approximately 250 000 variants in each of the three families. After initial filtering of the exome sequence data, 28, 37 and 24 candidate gene mutations remained in the three families. Validation, high-throughput genotyping of large numbers of Australian control samples and further filtering based on extended public SNP databases reduced candidate gene numbers to 13, 29 and 11(5). Presently, data from protein predictions and gene function analysis is being collated to prioritise candidates for functional studies.

Discussion and conclusion: The genetic defects remain to be identified in around 38% of Australian familial ALS cases in our cohort. The identification of novel gene mutations and characterisation of their functional consequences will provide further insight into the genetic and pathological basis of motor neuron degeneration, providing new targets for diagnosis and therapeutic development. Furthermore, novel gene mutations will lead to the development of new *in vitro* and *in vivo* disease models, which may provide a platform for the full understanding of ALS pathogenesis.

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P172 PROJECT MINE PHASE 1: IMPUTATION OF ALS GENOMES IN A GENOME WIDE ASSOCIATION STUDY

CONSORTIUM MINE

University Medical Center Utrecht, Utrecht, The Netherlands

Email address for correspondence: w.vanrheenen-2@umcutrecht.nl

Keywords: GWAS, sequencing, imputation

Background: Genetic risk factors play an important role in ALS susceptibility. So far, genome wide association studies (GWAS) have identified 3 genetic risk factors (*C9ORF72, UNC13A* and a locus on 17q11.2). The *C9ORF72* repeat expansion, being the most common cause of ALS, exhibits a clear founder effect and virtually all patients carrying the repeat expansion share a common haplotype at chromosome 9p21.2. Including 6,100 cases and 7,125 controls in the most recent GWAS meta-analysis, these studies are only moderately powered according to GWAS standards. To find new genetic risk loci in ALS we are conducting well powered GWAS and will improve imputation of ALS specific haplotypes by enriching our reference panel with high coverage whole genome sequencing data of 1,250 ALS patients.

Methods: Raw genotype data for all strata in previously published GWAS's in ALS were obtained. In addition 7,603 new patients and 3,811 new control subjects were genotyped on the Illumina OmniExpress array. Standard GWAS QC-measures for SNPs and individuals were performed per stratum and for all data combined. For the reference panel whole genome sequencing data at an average coverage of 40X was obtained for Dutch ALS patients and matched controls. After quality control this ALS-specific reference panel was phased using SHAPEIT2 software and was merged with publicly available reference panels (ie, 1000 Genomes, GoNL and UK10K). The GWAS strata will be imputed with the combined reference panel using IMPUTE2 and meta-analysed. To assess imputation accuracy masked whole genome sequencing data from Dutch familial ALS patients and trios were imputed using the different reference panels.

Results: In total 14,668 patients and 24,812 controls were included from 16 different nationalities. Final QC measures are currently assessed and the data will be prepared for imputation. When assessing the imputation accuracy, our ALS-specific reference panels is superior to 1000 Genomes and GoNL. Even for very rare alleles (<0.5%) that are notoriously hard to impute concordance between imputed and sequenced genotypes of 74.6% is achieved using the ALS-genomes as reference panel compared to 69.6% and 47.1% for GoNL and 1000 Genomes respectively. When combining all reference panels the performance can only be improved by allowing a large number of haplotypes for imputation (78.7% when using 5000 haplotypes). Results on the association analysis and therefore possible new risk loci will be presented if available.

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P173 AN UPDATE ON THE ALS ONLINE GENETICS DATABASE, ALSOD

ABEL O[1], POWELL JF[3], ANDERSEN P[2], AL-CHALABI A[1]

[1]*Department of Clinical Neuroscience Institute of Psychiatry, London, UK* [2]*Department of Pharmacology and Clinical Neuroscience, Section for Neurology, Umeå University, Umea, Sweden,* [3]*Department of Neuroscience, King's College London, London, UK*

Email address for correspondence: olubunmi.k.abel@kcl.ac.uk

Keywords: genetics, bioinformatics, ALSoD

Amyotrophic lateral sclerosis, also known as Lou Gehrig's disease, typically leads to death within 3–5 years of symptom onset. Understanding what causes ALS is an obstacle, but more research in this area, enhanced by advanced technology like high-throughput and next generation sequencing, is paving the way for better information and direction. The volume of data generated by genetics researchers has dramatically increased, largely because of increased opportunities for collaboration. ALSoD, a widely used online genetics database for collating, analysing and integrating ALS data, has been updated, with analytics tools able to portray the data graphically to users.

Mutations and other gene variants have been mapped to genomic coordinates, and the inclusion of dbSNP IDs has been implemented to facilitate the integration of data from

numerous public sources. To increase the usability and functionality of ALSoD, population frequency of each variant found in the 1000 genome and EVS databases is displayed. To contribute to a better understanding of the pathogenesis of ALS, links to information on animal models are also available.

The database can now be viewed on mobile devices and for Android platforms, a mobile app is available. A more attractive genetic website can be built without extra expenses ie, changing the whole programming platform. A page to calculate the penetrance of the disease in a family is also included in the new version. Using the pdb ID, a user can interact with the molecular structures of mutations on the corresponding webpages.

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P174 TWO ALS CASES CARRYING A NOVEL P. Q121G MISSENSE MUTATION IN EXON 5 OF SOD1 GENE

TATEISHI T[1,2], HAYASHI S[2], IINUMA K[2], MURAI H[2], KIRA J-I[2]

[1]*Department of Neurology, Iizuka Hospital, Iizuka, Japan,* [2]*Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan*

Email address for correspondence: tatetaka@neuro.med.kyushu-u.ac.jp

Keywords: SOD1, Q121G, mutation

Background: Genetically, 20% of FALS cases carry missense mutations in the SOD1 gene. 177 mutations have been reported to date.

Objectives: To describe two cases with a novel Q121G missense mutation in the SOD1 gene.

Methods: We carried out clinical and genetic studies on two Japanese patients with the SOD1 gene (Q121G) mutation.

Case reports: Patient 1 suffered from weakness of the right leg at age 69. Over the following year, the weakness spread to his left lower limb, leading to gait disturbance. He was admitted to our hospital at the age of 70. He was born the eighth of nine children, three of whom suffered from ALS in adult life. Neurological examination on admission revealed a proximal dominant moderate weakness in the bilateral lower limb muscles. Deep tendon reflexes were exaggerated in the upper limbs, and attenuated or lost in the lower limbs. The planter response was extensor on both sides. Vibration sense was moderately decreased in the lower extremities and urinary disturbance was noted. Muscle atrophy of tongue, dysphagia and dysarthria were not observed. EMG showed chronic neurogenic patterns, including giant motor unit potentials in the four limbs. Two years after onset, dysphagia appeared, and non-invasive ventilation was initiated because of decreased vital capacity. Five years after the onset, the respiratory disturbance became worse, resulting in death at 74 years of age.

Patient 2 noticed muscle weakness in his left foot at 66 years of age, which progressively deteriorated during the following two years. He developed urinary disturbance three years after the onset, and was admitted to our hospital. He has no family history of ALS. At admission, neurological examination demonstrated muscle weakness, fasciculation and atrophy of the bilateral lower limb, hyperreflexia in both upper limbs, and hyperreflexia in both lower limbs. The plantar response was flexor. Vibration sense was moderately decreased in the lower extremities and urinary disturbance was observed. Muscle atrophy of tongue, dysphagia and dysarthria were not observed. EMG showed acute neurogenic patterns in left upper limb and acute and chronic neurogenic patterns in left lower limb. The gait disturbance progressed, and the patient was unable to walk unaided four years after onset.

Molecular analysis showed the heterozygous (patient 1) and homozygous (patient 2) missense mutation c.362 A>G; p. Q121G was found in exon 5 of SOD1 gene.

Discussion and conclusion: To our knowledge, no other missense mutation of codon 121 of SOD1 has been found. These cases showed moderately decreased vibration sense in the lower extremities and urinary disturbance at an earlier stage of the disease. These symptoms may be a characteristic feature of this mutation, but we need further data to establish a genotype – phenotype correlation.

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P175 DELETERIOUS VARIATIONS IN THE ESSENTIAL MRNA METABOLISM FACTOR, HGLE1, IN ALS PATIENTS

KANEB HM[1], FOLKMANN AW[2], BELZIL VV[3], JAO L-E[2], LEBLOND CS[1], GIRARD SL[3], DAOUD H[3], NOREAU A[3], ROCHEFORT D[1], HINCE P[1], LEVERT A[1], VIDAL S[3], ANDRÉ-GUIMONT C[3], CAMU W[4], BOUCHARD J-P[5], DUPRÉ N[5], ROULEAU GA[1], WENTE SR[2], DION PA[1,3]

[1]*McGill University, Montreal, Quebec, Canada,* [2]*Vanderbilt University School of Medicine, Nashville, Tennessee, USA,* [3]*Université de Montréal, Montreal, Quebec, Canada,* [4]*Unité de Neurologie Comportementale et Dégénérative, Institute of Biology, Montpellier, France,* [5]*Laval University, Centre Hopitalier Universitaire de Québec, Quebec City, Quebec, Canada*

Email address for correspondence: hannah.kaneb@mail.mcgill.ca

Keywords: Gle1, RNA processing, genetics

Background: Causative mutations in the global RNA processing proteins TDP-43 and FUS (amongst others) as well as their aggregation in ALS patients have identified defects in RNA metabolism as an important feature of this disease. Lethal congenital contracture syndrome 1 (LCCS1) and lethal arthrogryposis with anterior horn cell disease (LAAHD) are autosomal recessive fetal motor neuron diseases that are caused by mutations in another global RNA-processing protein, hGle1(1).

Objectives: To test the hypothesis that mutations in hGle1 are causative for ALS as well as for LCCS1/LAAHD

Methods: 173 unrelated familial ALS cases (FALS), 760 sporadic ALS (SALS) cases and 190 matched controls, of European descent, were screened for mutations in *GLE1* via Sanger sequencing. Exons in which variants were identified were sequenced via the Sanger method in 285 further controls. All variants identified were then investigated in an independent whole exome sequencing dataset of 485 matched controls with no known neurodegenerative disorders.

Results: Although no excess of *GLE1* variations was identified in ALS patients over controls we observed a striking

absence of deleterious variations in controls and conversely identified two deleterious variations in ALS patients (one splice site (FALS) and one nonsense (SALS) variation). Functional analyses of these deleterious variations revealed them to be unable to rescue motor neuron pathology in zebrafish morphants lacking Gle1. Furthermore, in HeLa cells both mutations caused a depletion of hGle1 at the nuclear pore where it carries out an essential role in nuclear export of mRNA as well as reduced overall levels of hGle1 in the cytoplasm.

Discussion and conclusion: These results suggest a haploinsufficiency mechanism and highlight *GLE1* as a strong candidate for a causative gene for ALS. This further supports the involvement of global defects in RNA metabolism in this disease.

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P176 CHARACTERING NOVEL ALTERNATIVELY SPLICED ISOFORMS OF THE EPHA4 GENE

MANGELSDORF M[1], ZHAO J[1], NARAYANAN RK[1], BUTLER TJ[1], HENDERSON RD[2], MCCOMBE PA[2], BARTLETT PF[1]

[1]*Queensland Brain Institute, The University of Queensland, Queensland, Australia,* [2]*Department of Neurology, Royal Brisbane and Women's Hospital, Queensland, Australia*

Email address for correspondence: m.mangelsdorf@uq.edu.au

Keywords: EPHA4, modifier, TDP-43

Background: Alternative splicing is a common and essential phenomenon in human genes, and disruption of the process is associated with human genetic disease. There are currently > 20 genes in which mutations cause amyotrophic lateral sclerosis (ALS). A growing number of these play a role in RNA metabolism, including pre-mRNA splicing. We have identified EPHA4 as one gene whose pre-mRNA splicing is regulated by TDP-43.

Objectives: Given that EPHA4 has been implicated as a modifier of ALS and is regulated by TDP-43 we aimed to characterise alternative isoforms of EPHA4 and determine the role they play in ALS.

Methods and results: Analysis of the expressed sequence tags from the UniGene clusters of both mouse and human EPHA4 revealed the presence of possible novel isoforms. Reverse-transcriptase PCR validated the presence of two of the novel isoforms in both human and mouse brain and spinal cord. To determine if these isoforms encode protein we have performed Western blot hybridization using EPHA4 antibodies and have identified proteins consistent with the novel isoforms in mouse tissue. Expression of the novel isoforms in HEK293T cells has shown a similar localisation to the plasma membrane as the full length EPHA4 for one isoform, however the second shows a novel diffuse cytoplasmic localisation.

Discussion and conclusion: We have identified novel coding mRNA isoforms transcribed from the EPHA4 gene. We are now assessing the effect of the new isoforms on EPHA4 activation. Investigating the functional significance of these novel isoforms will be important to fully understand the contribution of EPHA4 to disease progression in ALS, and thus which may be a targets for disease therapy.

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P177 SAITOHIN GENE RS62063857 VARIANT IN SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

SONMEZ E[1], SAZCI A[1], OZEL MD[1], IDRISOGLU HA[2]

[1]*University of Kocaeli, Faculty of Medicine, Department of Medical Biology and Genetics, Kocaeli, Turkey,* [2]*University of Istanbul, Istanbul Faculty of Medicine, Department of Neurology, Istanbul, Turkey*

Email address for correspondence: alisazci@gmail.com

Keywords: sALS, saitohin (STH), rs62063857

Background: Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron disease (MND), affecting either upper or lower motor neurons. Generally patients experience signs and symptoms of progressive muscle atrophy and weakness, problems with swallowing, leading to respiratory failure and death. Only 5–10% of patients survive more than 10 years and median survival is 3–5 years from onset (1).

Objects: The aim of the study was to determine whether the saitohin (STH) gene rs62063857 variant is associated with sporadic amyotrophic lateral sclerosis (sALS).

Methods: We had 597 sporadic ALS patients and 423 controls to genotype. Genomic DNA was isolated from whole blood and a polymerase chain reaction and restriction fragment length polymorphism procedure was used to genotype the STH gene rs62063857 variant (2). The amplified fragment was digested HifI restriction endonuclease and run on an 8% polyacrylimide gel followed by silver staining. SPSS statistical analysis was used in the calculation of genotype and allele frequencies and association.

Results: The satiohin gene rs62063857 variant was not associated with SALS ($\chi^2 = 0.063$, $P = 0.969$). The AA, AG and GG genotype frequencies in sALS were 61.5, 34.0 and 4.5% in cases and 60.8, 34.5 and 4.7% in controls respectively. The A and G allele frequencies were 78.48 and 21.52% in cases and 78.0 and 22.0% in the controls respectively. There was no association on gender ($\chi^2 = 0.345$, $P = 0.842$ in male and $\chi^2 = 1.113$, $p = 0.573$ in female).

Discussion and conclusion: Although ALS is a rare disease with a prevalence of 1–2 in 100,000 worldwide, it is a devastating disorder. In our earlier studies, we found that STH rs62063857 variant was associated with dementia (3) and Parkinson's disease. In this particular study, we did not find any association between the STH gene rs62063857 variant and SALS. In this study, the hypothesis was that STH gene rs62063857 variant could be used as a biomarker to differentiate SALS from Dementia and Parkinson's Disease

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P178 CX3CR1 IS A MODIFYING GENE OF SURVIVAL AND PROGRESSION IN AMYOTROPHIC LATERAL SCLEROSIS

SALVADO M[1], LOPEZ-LOPEZ A[2], SYRIANI E[1], MORALES M[1], RODRÍGUEZ MJ[2], CAZORLA S[1], MAHY N[2], VIDAL-TABOADA JM[2], GAMEZ J[1]

[1]*ALS Unit, Neuromuscular Disorders Clinic, VHIR. UAB, Barcelona, Spain,* [2]*Biochemistry and Molecular Biology Unit, Department of Physiological Sciences I, Faculty of Medicine - IDIBAPS, University of Barcelona, Barcelona, Spain*

Email address for correspondence: josepgamez.bcn@gmail.com

Keywords: CX3CR1, influencing prognostic factors, modifying gene

Background: The reason why some ALS patients deteriorate much faster or survive much longer than others is unknown. An important challenge for ALS research is to determine how endogenous factors modify the disease to account for these different disease courses. The discovery of new biomarkers associated with different rates of progression and survival could provide new insights into the pathophysiological determinants of disease progression. Microglia activation and the crosstalk between immune cells appear to play a significant role in neuronal death. Microglial activation may modify disease progression and symptoms, and therefore disease outcome.

Objective: To investigate the association of functional variants of the human CX3CR1 gene (Fractalkine receptor) with the risk of ALS, the survival and the progression rate of the disease symptoms in a Spanish ALS cohort.

Methods: 187 ALS patients (142 sALS and 45 fALS) and 378 controls were recruited. We investigated CX3CR1 V249I (rs3732379) and T280M (rs3732378) genotypes and their haplotypes as predictors of survival, the progression rate of the symptoms (as measured by ALSFRS-R and FVC decline) and the risk of suffering ALS disease.

Results: The sALS patients with CX3CR1 249I/I or 249V/I genotypes presented a shorter survival time (42.2764.90) than patients with 249V/V genotype (67.6567.42; diff 225.49 months 95%CI (242.79, 28.18); p = 0.004; adj-p = 0.018). The survival time was shorter in sALS patients with spinal topography and CX3CR1 249I alleles (diff = 229.78 months; 95%CI (249.42, 210.14); p = 0.003). The same effects were also observed in the spinal sALS patients with 249I–280M haplotype (diff = 227.02 months; 95%CI (249.57, 24.48); p = 0.019). In the sALS group, the CX3CR1 249I variant was associated with a faster progression of the disease symptoms (OR = 2.58; 95%CI (1.32, 5.07); p = 0.006; adj-p = 0.027).

Discussion and conclusion: The progression rate of the disease symptoms and the survival time is affected in ALS patients with one or two copies of the CX3CR1 249I allele. The association evidenced herein is clinically relevant and indicates that CX3CR1 could be a disease-modifying gene in sALS.

Conclusion: The CX3CR1 is the most potent ALS survival genetic factor reported to date. These results reinforce the role of the immune system in ALS pathogenesis.

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P179 INVOLVEMENT OF HOMEOBOX GENES IN C9ORF72-RELATED DISEASES

VAN BLITTERSWIJK M[1], WANG X[2], ASMANN Y[2], BAKER M[1], BROWN P[1], JOSEPHS K[3], PARISI J[3], KNOPMAN D[3], PETERSEN R[3], BOEVE B[3], GRAFF-RADFORD N[4], BOYLAN K[4], RADEMAKERS R[1]

[1]*Department of Neuroscience,* [2]*Department of Health Sciences Research,* [3]*Department of Neurology,* [4]*Department of Neurology, Mayo Clinic Jacksonville, FL, USA*

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

Keywords: C9ORF72, homeobox genes

Background: A GGGGCC-repeat expansion in chromosome 9 open reading frame 72 (*C9ORF72*) is the most common genetic cause of frontotemporal dementia (FTD) and motor neuron disease (MND). Although previous studies have revealed reduced expression of *C9ORF72* and the formation of RNA foci and dipeptide-repeat proteins, it is unclear how this eventually leads to neurodegeneration.

Objective: To elucidate the mechanisms underlying *C9ORF72*-related diseases we investigated the differential expression, alternative splicing and methylation profiles of individuals with *C9ORF72* repeat expansions.

Methods: Thirty-two *C9ORF72* expansion carriers were investigated (12 FTD, 10 FTD/MND and 10 MND) as well as 30 disease controls (10 FTD, 10 FTD/MND and 10 MND) and 20 controls without neurological disorders. The Whole-Genome DASL HT Assay was used to assess expression profiles, separately for the frontal cortex and cerebellum. Differentially expressed genes were identified using the lumi R package and analyzed with enrichment and network modules (MetaCore). To further investigate the frontal cortex we performed RNA-Seq analysis for 8 *C9ORF72* expansion carriers (5 FTD and 3 FTD/MND) and 8 disease controls (6 FTD and 2 FTD/MND), including differential expression (DESeq2) and alternative splicing (multivariate analysis of transcript splicing (MATS)) analyses. We also studied genome-wide methylation profiles in the cerebellum of 15 *C9ORF72* expansion carriers (5 FTD, 5 FTD/MND and 5 MND) in addition to 15 disease controls (5 FTD, 5 FTD/MND and 5 MND) and 15 controls; the Infinium Human Methylation450 BeadChip was used and data was analyzed with the lumi R package.

Results: In the cerebellum, we identified 40 differentially expressed genes that remained significant after FDR correction when comparing *C9ORF72* expansion carriers to disease controls (eg, homeobox genes). Importantly, we observed enrichment for gene ontology (GO) processes involved in development (eg, organ morphogenesis and skeletal system development (FDR < 5E-09)). In the frontal cortex, only three genes were differentially expressed after correction for multiple testing, including homeobox genes and *C9ORF72*. All findings were comparable when focusing on disease subgroups, and when comparing cases to controls without neurological diseases; the upregulation of homeobox genes was validated with quantitative real-time PCR. To further investigate our findings, we also examined alternative splicing and methylation profiles, which provided additional evidence for the essential role of developmental processes (eg, 460 skipped exon events; enrichment for nervous system development and neuron projection morphogenesis (FDR < 5E-09)).

Discussion and conclusion: We are the first to describe involvement of homeobox genes in *C9ORF72*-related diseases, as supported by DASL arrays, RNA-Seq, and Methylation450 BeadChips. Based on our results, we speculate that homeobox genes might be a key player in the degeneration of neurons observed in *C9ORF72* expansion carriers. Our findings, therefore, increase our understanding of *C9ORF72*-related diseases and reveal interesting targets for novel treatment strategies.

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P180 A MITOCHONDRIAL ORIGIN FOR FRONTOTEMPORAL DEMENTIA AND AMYOTROPHIC LATERAL SCLEROSIS THROUGH CHCHD10 INVOLVEMENT

BANNWARTH S[1,2], AIT-EL-MKADEM S[1,2], CHAUSSENOT A[1,2], GENIN EC.[1], LACAS-GERVAIS S[3], FRAGAKI K[1,2], BERG-ALONSO L[1], KAGEYAMA Y[4], SERRE V[5], MOORE D[6], VERSCHUEREN A[7], ROUZIER C[1,2], LE BER I[8,9], LESPINASSE F[1], N'GUYEN K[10], BRICE AS[8], YU-WAI-MAN P[6,11], SESAKI H[4], POUGET J[7], PAQUIS-FLUCKLINGER V[1, 2]

[1]*IRCAN, UMR CNRS 7284/INSERM U1081/UNS, School of Medicine,* [2]*Department of Medical Genetics, National Centre for Mitochondrial Diseases,* [3]*Joint Center for Applied Electron Microscopy, Nice Sophia-Antipolis University, Nice, France,* [4]*Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, USA,* [5]*UMR7592 CNRS, Jacques Monod Institute, Paris Diderot University, Paris, France,* [6]*Wellcome Trust Centre for Mitochondrial Research, Institute of Genetic Medicine, International Centre for Life, Newcastle University, Newcastle, UK,* [7]*Department of Neurology, Timone Hospital, Marseille, France,* [8]*Sorbonne Université, UPMC Univ Paris 06, UM75, Inserm U1127, Cnrs UMR7225, Nice, France,* [9]*National Reference Centre on Rare Dementias, AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Paris, France,* [10]*Department of Medical Genetics, Timone Hospital, Marseille Teaching Hospital, Marseille, France,* [11]*Newcastle Eye Centre, Royal Victoria Infirmary, Newcastle upon Tyne, Newcastle, France*

Email address for correspondence: chaussenot.a@chu-nice.fr

Keywords: FTD-ALS, CHCHD10, mitochondrial DNA instability

Background: Mitochondrial DNA (mtDNA) instability disorders are responsible for a large clinical spectrum, among which amyotrophic lateral sclerosis-like symptoms and frontotemporal dementia are extremely rare.

Objectives: We report a large family with a late-onset phenotype including motor neuron disease, cognitive decline looking like frontotemporal dementia, cerebellar ataxia and myopathy.

Results: In all patients, muscle biopsy showed ragged-red and COX negative fibres with combined respiratory chain deficiency and abnormal assembly of complex V. The multiple mtDNA deletions found in skeletal muscle revealed a mtDNA instability disorder. Patient fibroblasts present with respiratory chain deficiency, mitochondrial ultrastructural alterations and fragmentation of the mitochondrial network. Interestingly, expression of matrix-targeted photoactivable GFP showed that mitochondrial fusion was not inhibited in patient fibroblasts. By whole-exome sequencing (WES), we identified a missense mutation (c.176C>T; p.Ser59Leu) in the *CHCHD10* gene that encodes a coiled-coil helix protein, whose function is unknown. We show that CHCHD10 is a mitochondrial protein located in the intermembrane space and enriched at cristae junctions. Overexpression of *CHCHD10* mutant allele in HeLa cells led to fragmentation of the mitochondrial network and ultrastructural major abnormalities including loss, disorganization and dilatation of cristae. The observation of a frontotemporal dementia-amyotrophic lateral sclerosis (FTD-ALS) phenotype in a mitochondrial disease led us to analyse *CHCHD10* in a cohort of 21 families with pathologically proven FTD-ALS. We identified the same missense p.Ser59-Leu mutation in one of these FTD-ALS families.

Discussion and conclusion: This work opens a novel field to explore the pathogenesis of FTD-ALS clinical spectrum by showing that mitochondrial disease may be at the origin of some of these phenotypes.

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THEME 8 HUMAN CELL BIOLOGY AND PATHOLOGY

P181 SERUM SIGNATURES OF MICRORNAS IN GENETIC ALS PATIENTS AND PRE-MANIFEST MUTATION CARRIERS

FREISCHMIDT A[1], MÜLLER K[1], ZONDLER L[1], WEYDT P[1], VOLK AE[1], LOŠDORFER BOŽIC A[2], WALTER M[3], BONIN M[3], MAYER B[1], OTTO M[1], DIETERICH C[2], HOLZMANN K[1], ANDERSEN P[1,4], LUDOLPH AC[1], DANZER KM[1], WEISHAUPT JH[1]

[1]*Ulm University, Ulm, Germany,* [2]*Max Planck Institute for Biology of Ageing, Cologne, Germany,* [3]*University of Tübingen, Tübingen, Germany,* [4]*Umeå University, Umeå, Sweden*

Email address for correspondence: axel.freischmidt@web.de

Keywords: microRNA, mutation carrier, serum

The nature and timepoint of patho-molecular alterations preceding onset of symptoms in amyotrophic lateral sclerosis (ALS) is unknown, but might govern future concepts of pre-manifest disease modifying treatments. MicroRNAs (miRNAs) are central regulators of transcriptome plasticity and participate in pathogenic cascades and/or mirror cellular adaptation to insults. We obtained comprehensive expression profiles of miRNAs in the serum of familial ALS patients (fALS), asymptomatic mutation carriers and healthy controls. We observed a strikingly homogenous miRNA profile in fALS patients that was largely independent from the underlying disease gene. Moreover, we identified 24 significantly downregulated miRNAs in pre-manifest ALS mutation carriers up to 20 years before the estimated time window of disease onset. 91.7% of miRNA alterations in mutation carriers overlapped with the fALS patients. Our data thus suggest specific common denominators regarding molecular pathogenesis of different ALS genes. We describe the earliest pathomolecular alterations in ALS mutation carriers known to date, which provide a basis for the discovery of novel therapeutic targets and strongly argue for studies evaluating pre-symptomatic disease-modifying treatment in ALS.

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P182 THE INFLUENCE OF NEUROFILAMENT PROTEINS ON TRAFFICKING OF RNA BINDING PROTEINS LINKED TO ALS

DURHAM H, TIBSHIRANI M, GENTIL B

McGill University, Montreal, QC, Canada

Email address for correspondence: heather.durham@mcgill.ca

Keywords: RNA binding proteins, neurofilaments, preferential vulnerability

Background: The high content of neurofilaments (NFs) in motor neurons, including NF bundles in dendrites, is a factor in their preferential vulnerability to disease. The relative expression of NF proteins (NFL, NFM, NFH, peripherin) is altered in sporadic ALS and is influenced by mutant proteins associated with familial disease, including SOD1 and RNA-binding proteins such as TDP-43, which decrease stability of *NEFL* mRNA (1). Trafficking of RNA binding proteins can be altered in both sporadic and familial forms of ALS, leading to their accumulation in cytoplasmic inclusions in affected neurons. This finding is reproduced in cultured motor neurons ectopically expressing these proteins (2, 3).

Objectives: In this study, we tested the hypothesis that NFs promote cytoplasmic accumulation and aggregation of ALS-associated mutant RNA binding proteins, TDP-43 and FUS, thereby contributing to preferential vulnerability of motor neurons to dysfunction.

Methods: Spinal cord-DRG cultures were prepared from E13 embryos of $Nefl^{-/-}$ mice or wild type $Nefl^{+/+}$ mice. NFL is a core protein required for NF assembly, thus $Nefl^{-/-}$ mice lack NFs. Plasmids encoding flag-tagged human FUS^{WT}, FUS^{R521H}, TDP-43^{WT}, or TDP-43^{A315T} were expressed in motor neurons of mature cultures by intranuclear microinjection. Distribution of endogenous or ectopically expressed protein was assessed by immunocytochemistry after three days and the distribution was quantified as nuclear, cytoplasmic or both. NF formation was induced in $Nefl^{+/+}$ neurons by expressing NFL.

Results: Distribution of mutant FUS and TDP-43 was much more nuclear in $Nefl^{-/-}$ compared to $Nefl^{+/+}$ motor neurons, the percentage of neurons with cytoplasmic protein and inclusions being reduced by about 50% in $Nefl^{-/-}$ neurons ($p < 0.05$). This was not due to variability among cultures as cytoplasmic protein and inclusions were increased in $Nefl^{-/-}$ neurons by ectopically expressing NFL. Interestingly, expression of NFL^{Q333P}, a mutant causing Charcot-Marie-Tooth disease 2E, promoted redistribution of endogenous FUS and TDP-43 from the nucleus to the cytoplasm, affirming the influence of NF proteins on distribution of RNA binding proteins and their involvement in pathogenesis of multiple disorders affecting motor neurons.

Discussion and conclusions: These data reinforce NFs as a factor in preferential vulnerability of motor neurons to disease, at the mRNA, protein, and structural level. NF proteins affect a wide variety of cellular processes through structural and signalling mechanisms, including common pathways affected in multiple forms of ALS (inclusion formation, mitochondrial dynamics, calcium handling, RNA metabolism, dendritic structure/function, and response to stress).

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P183 OPTINEURIN AND MYOSIN VI-RELATED TRAFFICKING DEFECTS IN SPORADIC ALS

SUNDARAMOORTHY V, WALKER AK, FARG M[2], SOO KY[2], ATKIN J[1,2]

[1]*Australian School of Advanced Medicine, Macquarie University, NSW, Australia,* [2]*Department of Biochemistry, La Trobe University, Victoria, Australia*

Email address for correspondence: julie.atkin@mq.edu.au

Keywords: myosin VI, autophagy, optineurin

Background: Defects in vesicular trafficking are increasingly implicated in ALS. Mutations in optineurin cause a proportion of familial ALS cases, and wild type optineurin is misfolded and forms inclusions in sporadic ALS motor neurons. However it is unknown how optineurin mutation or misfolding leads to ALS. Optineurin acts an adaptor protein connecting the molecular motor myosin VI to secretory vesicles and autophagosomes.

Objectives: To (i) determine how optineurin mutation leads to ALS and (ii) determine whether the same defects are present in sporadic ALS.

Methods: Autophagy was examined using immunoblotting and immunocytochemistry. Sporadic ALS patient tissues were examined by immunohistochemistry and immunoblotting.

Results: Here we demonstrate that ALS-linked mutations Q398X and E478G cause optineurin to dissociate from myosin VI, leading to an abnormal diffuse cytoplasmic distribution rather than the normal vesicular appearance. Furthermore, in control human patient tissues optineurin displayed its normal vesicular localization but in sporadic ALS patient tissues, vesicles were present in a significantly reduced proportion of motor neurons. In addition, optineurin binding to myosin VI was decreased in tissue lysates from sporadic ALS spinal cords, implying that disruption of optineurin-myosin VI function is present in sporadic as well as familial disease.

Expression of Q398X and E478G mutant optineurin in motor neuron-like NSC-34 cells also inhibited secretory protein trafficking, induced endoplasmic reticulum (ER) stress and caused Golgi fragmentation. These defects were not present in control NSC-34 cells expressing another optineurin mutation, E50K, which causes primary open angle glaucoma. We also provide further insight into the role of optineurin as an autophagy receptor. Wildtype optineurin associated with lysosomes and promoted autophagosome fusion to lysosomes in neuronal cells, implying it mediates trafficking of lysosomes during autophagy in association with myosin VI. However, either expression of ALS mutant optineurin in these cells, or siRNA-mediated knockdown of endogenous optineurin, blocked lysosome fusion to autophagosomes, resulting in autophagosome accumulation.

Discussion and conclusion: Together these results indicate that ALS-linked mutations in optineurin disrupt myosin VI mediated intracellular trafficking processes. This study therefore links several previously described pathological mechanisms in ALS, including defects in autophagy, fragmentation of the Golgi, and induction of ER stress, to disruption of optineurin function. It also implies that optineurin-myosin VI dysfunction is a common and novel pathogenic mechanism of both sporadic and familial ALS.

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P184 EXTRACELLULAR VESICLES ARE IMPLICATED IN THE TRANSMISSION OF PROPAGATED SOD1 MISFOLDING IN ALS TISSUE

FERNANDO S, SILVERMAN J, GRAD L, CASHMAN N

University of British Columbia, Vancouver, BC, Canada

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

Keywords: protein misfolding, extracellular vesicles, SOD1

Background: A prion-like propagated protein misfolding mechanism has been observed for SOD1, which may help to explain the clinical observation that ALS disease pathology spreads spatiotemporally through the neuroaxis from one contiguous area to the next (1). We have previously shown that natively folded wild-type human SOD1 (WTSOD1) in cultured cells can be induced to misfold by physical contact with mutant misfolded SOD1 (misSOD1) (2), and that cell-to-cell transmission can occur between cells via the uptake of exosomes (3). However, the mechanism by which misSOD1 is propagated *in vivo* remains ambiguous.

Objective: To determine if extracellular vesicles (EVs), including exosomes, microvesicles, and other small secreted vesicle populations, are a mode of transportation for cell-to-cell propagation of misSOD1 in ALS mouse models and patient tissue, and to characterize the biology of EV populations from these tissues.

Methods: EVs were isolated from whole neuronal tissues harvested from transgenic mouse models of ALS or post-mortem tissue from ALS patients by dissociation with papain and serial centrifugation of resultant supernatant. Microvesicles and exosomes were isolated at separate centrifugal speeds, and exosomes were further purified in a sucrose density gradient. Both EV populations were examined via direct immunoblot to determine cargo and composition, and used in immunoprecipitation experiments to detect misSOD1.

Results: Isolated exosomes and microvesicles were positive for EV-specific markers such as prion protein, flotillin-1, and native WTSOD1. Immunoprecipitation using misSOD1-specific antibodies showed that misSOD1 is detected in EV fractions and is preferentially localized to the vesicle surface. Application of the secreted EV-containing fraction from cells onto wild-type cells in culture induced misfolding of SOD1 in those cells, a phenomenon which was abolished by heat-denaturing the EV-containing fraction prior to treatment of cells.

Conclusion: Pathogenic misSOD1 is present on EVs isolated from ALS patient and murine neuronal tissues and is abnormally localized to the surface of vesicles, whereas WTSOD1 is normally found in the lumen of EVs (4), suggesting that misSOD1 may be sorted into EVs in a manner different than WTSOD1. Our results also indicate that the secreted EV-containing fraction is competent to induce misfolding of WTSOD1 in cells, implicating the EV transport system in the propagation of SOD1 misfolding seen in disease.

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P185 RNA METABOLISM AND MITOCHONDRIAL DYSFUNCTION IN FIBROBLASTS FROM FAMILIAL ALS PATIENTS

COLOMBRITA C[1,2], ONESTO E[2], GUMINA V[2], DUSI S[3], BORGHI O[2], TIRANTI V[3], SILANI V[1,2], RATTI A[1,2]

[1]*Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy,* [2]*IRCCS Istituto Auxologico Italiano, Milan, Italy,* [3]*Fondazione IRCCS Istituto Neurologico "Carlo Besta", Milan, Italy*

Email address for correspondence: antonia.ratti@unimi.it

Keywords: RNA metabolism, mitochondria, fibroblasts

Background: Defects in RNA metabolism represent an important and still not well-defined pathogenic mechanism in ALS and frontotemporal dementia (FTD) in association to TDP-43 and FUS RNA-binding proteins, as well as to the recent discovered *C9ORF72* gene. Both loss- and gain-of function hypotheses have been suggested to explain the pathogenic role of these three genes in ALS and FTD. In particular, the loss of TDP-43 and FUS RNA-binding activities is supposed to greatly impair post-transcriptional regulatory mechanisms associated to pre-mRNA splicing, mRNA transport, stability and translation of target transcripts.

However, some recent literature data indicate that TDP-43 may also localize at mitochondria and that mitochondria functionality is altered in disease cellular models obtained by over-expressing both wild-type and mutant TDP-43. Mitochondrial dysmorphology and dysfunction have indeed been largely described in muscle and brain tissues from ALS patients and also in experimental cell and animal models expressing mutant *SOD1* gene. To date the potential link between RNA metabolism defects and mitochondrial activity has not been explored.

Objectives: To investigate whether mitochondrial function is altered in physiological disease cell models such as human fibroblasts derived from familial ALS patients carrying mutations in genes involved in RNA metabolism.

Methods: Primary fibroblasts were obtained from ALS patients carrying mutations in *TARDBP* and *C9ORF72* genes and from age- and sex-matched healthy controls. Mitochondrial morphology was analyzed by measuring specific parameters upon DsRedMito transfection. Several parameters referring to mitochondrial functionality and dynamics were analyzed and compared to control fibroblasts.

Results: Our results indicate that mitochondrial morphology and activity are altered both in *TARDBP*- and *C9ORF72*-mutated fibroblasts although in a different and gene-specific manner. Mitochondrial network appeared highly fragmented in mutant TDP-43 fibroblasts, as also sustained by changes in fission/fusion protein levels and in mitochondrial mass. Interestingly, over-expression of wild-type TDP-43 in mutant fibroblasts was able to rescue the observed mitochondrial morphology alterations. Mutant *TARDBP* and *C9ORF72* fibroblasts also exhibited different and specific alterations in mitochondrial activity by measuring mitochondrial membrane potential, respiration rate and ATP synthesis. Moreover, our immunofluorescence and sub-cellular fractionation data do not suggest that TDP-43 is localized at mitochondria and that it directly induces mitochondrial dysfunction in mutant *TARDBP*--expressing cells.

Discussion and conclusion: Mutant TDP-43 and *C9ORF72* may affect not only RNA metabolism, but also mitochondrial function in peripheral disease cell models obtained from familial ALS patients. Mutant fibroblasts may recapitulate the mitochondrial dysfunction occurring in affected neurons and therefore may represent a suitable disease cell models to study pathogenic mechanisms and for biomarker discovery and drug screening.

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P186 ALTERATIONS IN SARCO/ENDOPLASMIC RETICULUM PROTEINS IN HUMAN SKELETAL MUSCLE IN ALS AND OTHER NEUROPATHIC CONDITIONS

DERUSSO A[1], BILICILER S[3], KWAN J[2], CHIN E[1]

[1]*University of Maryland, College Park, MD, USA,* [2]*University of Maryland, Baltimore, MD, USA,* [3]*University of Texas Health Sciences Center, Houston, TX, USA*

Email address for correspondence: erchin@umd.edu

Keywords: skeletal muscle biomarkers, ER stress, Ca^{2+} regulation

Background: Recent studies in the $SOD1^{G93A}$ mouse model identified alterations in skeletal muscle intracellular Ca^{2+} regulation due to decreased expression of sarcoplasmic reticulum (SR) proteins (SERCA1 and SERCA2) (1). This was associated with increased expression of endoplasmic reticulum (ER) stress proteins (2) and proteins involved in regulating protein synthesis. These protein changes tracked with disease progression. Alterations in SR/ER function, possibly due to protein mis-folding, and the downstream changes in SR/ER protein expression may provide useful biomarkers of cellular pathology and disease progression in denervated muscle.

Objectives: To assess the changes in SR/ER proteins and markers of protein synthesis in skeletal muscle from ALS and other neuromuscular diseases.

Methods: Muscle biopsy samples were obtained from a tissue bank at the University of Texas Health Sciences Center in Houston. These samples were from normal (no evidence of neuromuscular disease, NORM; $n = 3$), disease control (myopathies, MYOP; $n = 3$) and ALS (ALS; $n = 3$) as determined by clinical evaluation and histopathology. Samples were homogenized and then analyzed for target protein expression by western blot. Images were quantified by densitometry using Image J.

Results: The SR Ca^{2+} pump protein SERCA1 was not different across groups and SERCA2 was increased in ALS 1.7

fold vs. NORM and 2.3-fold vs, MYOP. ER stress markers were also increased: GRP78/BiP by 6.8 -fold in ALS vs. NORM ($p < 0.05$) and CHOP 2.2 -fold in ALS vs. NORM and 2.1 -fold vs. MYOP ($p < 0.05$). GRP78/BiP was also elevated in MYOP vs. NORM (6.4 -fold; $p = 0.068$). Both total and phosphorylated p70S6K, markers of muscle hypertrophy and protein synthesis, were elevated in ALS (2.1 and 1.9 -fold, respectively, in ALS vs. NORM; $p < 0.05$) but unchanged in MYOP. The ratio of phosphorylated/total p70S6K, however, was decreased in ALS (to 90% vs. NORM; $p < 0.05$) and in MYOP (to 86% vs. NORM). Total Akt was also elevated in ALS (3-fold vs. NORM).

Discussion and conclusion: These preliminary data indicate that ER stress is activated in skeletal muscle under denervation conditions, with increased expression of chaperone protein GRP78/BiP and ER stress apoptotic marker CHOP. Markers of protein synthesis are also elevated suggesting possible futile cycling of protein synthesis and breakdown. SR Ca^{2+} handling proteins were not reduced, however, in contrast to the $SOD1^{G93A}$ model.

Conclusions: Quantitative analysis of muscle protein expression may be useful for discriminating between ALS and other myopathies.

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P187 UPREGULATION OF GLUTAMMINASE IN MICROGLIA OF SPINAL CORDS FROM SPORADIC ALS PATIENTS

INOSE Y[1], SHIBATA N[1], NIIDA-KAWAGUCHI M[1], KAKITA A[2]

[1]*Department of Pathology, Tokyo Women's Medical University, Tokyo, Japan,* [2]*Department of Pathology, Brain Research Institute, Niigata University, Niigata, Jordan*

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

Keywords: microglia; oxidative stress; tumor necrosis factor-α (TNF-α)

Background: Previous studies have suggested implications for inflammation (1) and excitotoxicity (2) in amyotrophic lateral sclerosis (ALS). Glutaminase (GLS) is an intracellular enzyme that catalyzes the deamination of glutamine to glutamate, and it has been shown to be upregulated in the presence of tumor necrosis factor-α (TNFα) (3).

Objectives: To determine the involvement of GLS in ALS.

Methods: Cervical segments of spinal cords from 10 sporadic ALS patients and 10 age-matched control subjects were examined. Formalin-fixed, paraffin-embedded or frozen sections of each case were used for immunohistochemistry, using specific antibodies to GLS (Abnova), TNFα (CST), ionized calcium-binding adaptor molecule 1 (Iba1) (Wako), glial fibrillary acidic protein (Dako), and N-methyl-D-aspartate receptor (NMDAR) (CST) . Antibody binding was visualized by the immunoperoxidase and immunofluorescence methods. Fresh materials of each case were used for immunoblotting, using antibodies to GLS and β-actin. Immunoreaction was detected by the chemiluminescence method, and optical densities of GLS-immunoreactive signals were statistically compared between the ALS and control groups by unpaired Student's t-test.

Results: Immunohistochemical analysis revealed that immunoreactivities for GLS and TNFα were mainly localized in Iba1-identified microglia, and staining was more intense in the ALS group. NMDAR immunoreactivity was localized in lower motor neurons. Immunoblot analysis disclosed a significant increase in β-actin-normalized GLS signals in the ALS group as compared to the control group ($p < 0.05$).

Discussion and conclusion: The present results suggest the involvement of TNFα-induced GLS upregulation in microglia and NMDAR-mediated glutamate neurotoxicity in ALS in an autocrine or paracrine manner.

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P188 IDENTIFICATION OF TRANSTHYRETIN AGGREGATES IN THE CSF OF ALS PATIENTS

BOWSER R[1], COLLINS M[2], AN J[1], PELLER D[1,3], LADHA S[1]

[1]*Barrow Neurological Institute, Phoenix, AZ, USA,* [2]*University of Pittsburgh, Pittsburgh, PA, USA,* [3]*Arizona State University, Tempe, AZ, USA*

Email address for correspondence: Robert.Bowser@DignityHealth.org

Keywords: cerebrospinal fluid, protein aggregation, biomarkers

Background: Many proteins have been identified within intracellular inclusions in motor neurons and glia of ALS patients. These include the RNA binding proteins TDP-43 and FUS, as well as SOD1. Misfolded SOD1 has also been proposed to be neurotoxic by cell-to-cell transfer via synaptic connections and exosome transport between cells. It may also be toxic via activation of neighboring microglia. We have a long standing interest investigating the CSF proteome in ALS patients, and have explored the possibility that misfolded or aggregated proteins may also exist in the CSF of ALS patients and contribute to the pathobiology of the disease.

Objectives: To identify aggregated proteins in the CSF of ALS patients and characterize potential neurotoxicity of these protein aggregates.

Methods: CSF was collected from ALS, healthy control and subjects with disease mimics, using our standard operating procedures for collection, processing and storage. The six most abundant proteins were removed from the CSF by column chromatography using HPLC (Agilent Hu-6HC column). The CSF samples were then fractionated by size exclusion chromatography on an Acquity UPLC (BEH200 SEC), and each fraction was analyzed by non-denaturing gel

electrophoresis. Western blot analysis was performed on the resulting blots, probing the blots with antibodies to proteins of interest. To examine potential neurotoxic nature of the protein aggregates, we cultured primary motor neurons generated from rat embryonic spinal cord. Protein aggregates were generated *in vitro* and incubated with cultured cells to examine effects on cell viability and cell morphology.

Results: We determined that transthyretin (TTR) exists in a high molecular weight aggregate in the CSF of approximately 75% of ALS patients. TTR aggregates were present in a very small proportion of healthy or disease control subjects. These results have been confirmed by filter binding assays.

Discussion and conclusions: We identified a novel extracellular protein aggregate in the CSF of ALS patients. Aggregation of TTR may disrupt its normal function and aggregated TTR has neurotoxic potential to motor neurons. TTR aggregates may also activate glia via cell surface receptors. Patients containing TTR aggregates did not have mutations in the TTR gene known to cause amyloidosis. It is possible that extracellular aggregates of TTR and other proteins may help facilitate spread of ALS to distal regions of the CNS. Our results highlight a novel route of disease pathogenesis/spread and a new therapeutic target.

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P189 ALS/FTLD WITH SQSTM1 MUTATIONS; LESSONS FROM PAGET'S DISEASE OF BONE

REA S[1], GOODE A[2,3], MAJCHER V[2], SEARLE M[3], LAYFIELD R[2]

[1]*Centre for Medical Research, University of Western Australia, and Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, Western Australia, Australia,* [2]*School of Life Sciences,* [3]*Centre for Biomolecular Sciences, School of Chemistry, University of Nottingham, Nottingham, UK*

Email address for correspondence: robert.layfield@nottingham.ac.uk

Keywords: SQSTM1, p62, autophagy

Background: Recently mutations affecting the *SQSTM1* gene, which encodes the p62/SQSTM1 protein, have been genetically associated with a small number of cases of ALS and FTLD. *SQSTM1* mutations have previously been identified as a common cause of the skeletal disorder Paget's disease of bone (PDB) and we have extensively studied them in this bone context. Significant coexistence of ALS/FTLD with PDB has not been recognized to date. However, although many mutations are ALS/FTLD-specific, several *SQSTM1* mutations are in fact common to both ALS/FTLD and PDB.

Objectives: To reassess how previous knowledge of the impact on p62/SQSTM1 function, of specific PDB-associated *SQSTM1* mutations which are now known to be relevant in ALS/FTLD, may provide new insights into ALS/FTLD disease mechanisms.

Methods: A literature analysis was performed to identify p62/SQSTM1-dependent pathways which *SQSTM1* mutations common to both PDB and ALS/FTLD impact upon, and also the functional significance of different domains of the p62/SQSTM1 protein which ALS/FTLD-specific mutations map to. Published data was reinterpreted in the context of previously proposed pathophysiological mechanisms in ALS/FTLD.

Results: *SQSTM1* mutations common to both PDB and ALS/FTLD have been reported to affect p62/SQSTM1-dependent NF-kappaB signalling, Keap1/Nrf2 signalling and autophagy pathways in various cell-based and animal model systems. Further, ALS/FTLD-specific mutations directly map to regions within the p62/SQSTM1 primary sequence shown to be essential for all three of these pathways. Changes associated with the three pathways are all consistent with proposed pathophysiological mechanisms in ALS/FTLD.

Discussion and conclusion: Investigation into the effects of disease-associated *SQSTM1* mutations on p62/SQSTM1-dependent pathways in model systems directly relevant to ALS/FTLD is now merited.

Acknowledgements: RL, MSS and AG are supported by the UK MND Association (Ref:6095).

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P190 PROTEIN DISULPHIDE ISOMERASE IS PROTECTIVE AGAINST THE PATHOLOGIES INDUCED BY MUTANT SOD1, TDP-43 AND FUS BOTH *IN VITRO* AND *IN VIVO* IN AMYOTROPHIC LATERAL SCLEROSIS

PARAKH S[1,2], SPENCER DM[2], HALLORAN M[1,2], ARACHCHILLAGE N[2], SOO KY[2], FARG M[2], WALKER AK[2], TURNER BJ[3], COLE NJ[1], ATKIN J[1,2]

[1]*Macquarie University, Sydney, NSW, Australia,* [2]*La Trobe University, Melbourne, Vic, Australia,* [3]*The Florey Institute, Melbourne, Vic, Australia*

Email address for correspondence: sonam.parakh@students.mq.edu.au

Keywords: ER stress, protein disulphide isomerase, SOD1

Background: Superoxide dismutase (SOD1), Fused in Sarcoma (FUS) and Tar-DNA binding protein-43 (TDP-43) are key proteins linked to amyotrophic lateral sclerosis (ALS) pathology. They share similar pathological hallmarks such as protein aggregation, inclusion formation, Endoplasmic Reticulum (ER) stress and ER-Golgi trafficking defects. Whilst neurodegenerative mechanisms are not fully defined in ALS, dysfunction to the ER is increasingly implicated in pathology (1). Protein disulphide isomerase (PDI) a key protein induced during ER stress, catalyses the formation of protein disulphide bonds and it also possesses general chaperone activity. It is primarily located in the ER but it is also found in other cellular locations. Our laboratory previously showed that over-expression of PDI is protective against mutant SOD1 pathology and a molecular mimic of PDI - BMC reduces SOD1 inclusions in neuronal cells(2). PDI also co-localises with FUS and TDP-43 positive inclusions in ALS patients. However, the mechanism of the protective action of PDI remains unclear.

Objectives: To investigate: (i) whether over-expression of PDI is protective against mutant FUS and TDP-43 cellular pathologies; (ii) how an ER chaperone can be protective

against mutant ALS proteins which are not generally present within the ER; (iii) the mechanisms underlying the protective activity of PDI; (iv) effect of PDI on transgenic FUS^{R521H} fish and effect of BMC on $SOD1^{G93A}$ mice.

Methods: Mutations in the active site of PDI were designed and expressed in motor neuronal cell lines with ALS-linked mutant proteins. Cellular pathologies were examined using immunofluorescence and immunocytochemistry. Transgenic zebra fish expressing FUS^{R521H} embryos were microinjected with constructs containing PDI-mkate and observed for FUS aggregation 24 h post fertilization. BMC was administered to $SOD1^{G93A}$ mice by ICV using mini osmotic pumps and counted at P100 (40 days infusion). Motor neurons in every third section were counted from a total of 30 ventral horns per mouse.

Results: PDI was protective against mutant FUS and TDP-43 induced pathology - ER stress, ER-Golgi trafficking defects and mis-localisation into cytoplasm. The disulphide activity, in contrast to its chaperone activity, was found to be the key factor necessary for its protective activity. However, we also found that the protective activity is enhanced when PDI is localised in the cytoplasm rather than the ER. Interestingly, PDI significantly decreased aggregation in FUS^{R521H} transgenic zebra fish. Furthermore, BMC was found to rescue motor neuron loss in $SOD1^{G93A}$ mice.

Conclusion: We demonstrate that PDI is protective against the major misfolded proteins linked to ALS. Hence small molecule mimics of PDI activity could be effective as a novel and broadly acting therapeutic agent in multiple forms of ALS.

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P191 THE IMMUNOMODULATORY EFFECTS OF HUMAN MESENCHYMAL STEM CELLS ON PERIPHERAL BLOOD MONONUCLEAR CELLS IN ALS PATIENTS

KWON M[2], NOH M-Y[1], OH K-W[1], CHO K-A[1], KANG B-Y[1], KIM K-S[3], KIM S-H[1]

[1]*Hanyang University, Seoul, Republic of Korea,* [2]*CHA University, Gyeonggi-do, Republic of Korea,* [3]*CoreStem Inc., Seoul, Republic of Korea*

Email address for correspondence: minsoo100@cha.ac.kr

Keywords: mesenchymal stem cell, regulatory T lymphocyte, cytokines

Background: In a previous study, we reported that central injection of MSCs slowed disease progression in $SOD1^{G93A}$ mice. In the present study, we found that central MSC administration vastly increased the infiltration of peripheral immune cells into the spinal cord of $SOD1^{G93A}$ mice. Thus, we investigated the immunomodulatory effect of MSCs on peripheral blood mononuclear cells (PBMCs) in ALS patients, focusing on regulatory T lymphocytes (T_{reg}; $CD4^+/CD25^{high}/FoxP3^+$) and the mRNA expression of several cytokines (IFN-γ, TNF-α, IL-17, IL-4, IL-10, IL-13 and TGF-β).

Methods: Peripheral blood samples were obtained from nine healthy controls (HC) and sixteen patients who were diagnosed with definite or probable ALS. Isolated PBMCs from the blood samples of all subjects were co-cultured with MSCs for 24h or 72h.

Results: Based on a FACS analysis, we found that co-culture with MSCs increased the T_{reg}/total T lymphocyte ratio in the PBMCs from both groups according to the co-culture duration. Co-culture of PBMCs with MSCs for 24h led to elevated mRNA levels of IFN-γ and IL-10 in the PBMCs from both groups. However, after co-culturing for 72h, although the IFN-γ mRNA level had returned to the basal level in co-cultured HC PBMCs, the IFN-γ mRNA level in co-cultured ALS PBMCs remained elevated. Additionally, the levels of IL-4 and TGF-β were markedly elevated, along with GATA mRNA, in the both HC and ALS PBMCs co-cultured for 72h. The elevated expression of these cytokines in the co-culture supernatant was confirmed via ELISA. Furthermore, we found that the increased mRNA level of indoleamine 2,3-dioxygenase (IDO) in the co-cultured MSCs was significantly correlated to the increase in Treg induction.

Conclusion: These findings of Treg induction and increased anti-inflammatory cytokine expression in co-cultured ALS PBMCs provide indirect evidence that MSCs may play a role in the immunomodulation of inflammatory responses when MSC therapy is targeted to ALS patients.

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P192 CALCIUM CHELATING ANTICOAGULANTS SIGNIFICANTLY AFFECT MONOCYTE/MACROPHAGE ACTIVATION ANTIGEN EXPRESSION IN ALS PATIENT BLOOD SPECIMENS

ZHANG R[1], MILLER RG[2], KATZ J[2], FORSHEW DA[2], MCGRATH MS[1]

[1]*University of California, San Francisco, San Francisco, California, USA,* [2]*California Pacific Medical Center, San Francisco, California, USA*

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

Keywords: immune activation; monocyte, anticoagulant

Background: Persistently activated monocytes expressing increased levels of HLA-DR and CD16 co-expressed are present in blood from patients with ALS. The degree of blood monocyle activation is correlated with plasma levels of LPS, a well-known monocyte activator, and is directly related to rate of disease progression in ALS. Our recent studies of ALS patient peripheral blood mononuclear cell gene expression showed upregulation of both M1 interferon-induced genes and M2 alternative activation genes, both consistent with a pattern of LPS activation. Together these data have led to the speculation that systemic immune system may play a significant role in ALS disease pathogenesis. However, there are discrepancies in the reported levels of monocyte activation in ALS (1,2). *In vitro* immune assays that require macrophage function can only be performed on heparinized blood, as calcium chelation with acid-citrate-dextrose (ACD) or EDTA blocks macrophage function.

Objectives: The goal of the current study was to test whether ACD, a calcium chelator that interferes wit

function, would also effect activation antigen expression as compared to a parallel evaluation of heparinized blood a form of anticoagulation that conserves macrophage function, in ALS.

Methods: 10 paired blood specimens from ALS patients were collected into two vacationer tubes containing either sodium heparin or ACD. Flow cytometry was performed to evaluate both T-cell and monocyte activation antigens.

Results: CD14 + monocytes in heparinized blood expressed significantly higher levels of HLA-DR (873.1 ± 244.6 vs. 423.4 ± 97.6, $p = 0.0020$) and CD16 (42.0 ± 11.2 vs. 10.5 ± 5.8, $p < 0.0001$) than monocytes in ACD blood. Monocytes expressing elevated HLA-DR and CD16 in heparinized blood showed significant increases in CD14-associated side scatter as a measure of granularity as compared to monocytes from ACD blood ($p = 0.0039$). A different pattern of anticoagulant effect was observed in analysis of T-cell activation. There were no differences in proportional levels of CD4 and CD8 T-lymphocyte subsets between heparinized and ACD blood. T-cell activation levels as quantified by detection of CD38 antigens on the surface of CD4 and CD8 T-cells was also similar between two anticoagulants.

Discussion and conclusion: The current study showed that two commonly used anticoagulants exerted various effects on expression of monocyte-inflammation/activation-related antigens. Significantly different levels of monocyte activation were observed between two anticoagulants. Monocytes which showed significantly increased levels of activation in heparinized blood had all of those disease associated levels revert to background when ACD was employed as the anticoagulant. T-cell markers were not different in either anticoagulant. As an anticoagulant, ACD works by chelating Ca^{2+}, which is essential for coagulation but also as a modulator of calcium-dependent monocyte activation. Our results suggest that special care must be taken in choosing an anticoagulant for a sample in which monocyte activation is to be evaluated.

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P193 NEURONAL LOSS IN FUNCTIONAL ZONES OF THE CEREBELLUM IN ALS

TAN R[1], MCGINLEY C[2], HASSANI M[1], KRIL J[2], KIERNAN MC[2], HORNBERGER M[1], HALLIDAY G[1]

[1]*Neuroscience Research Australia, NSW, Australia,* [2]*The University of Sydney, NSW, Australia*

Email address for correspondence: r.tan@neura.edu.au

Keywords: cerebellum, neurons, pathology

Introduction: The neuropathological hallmark of ALS is degeneration of upper and/or lower motor neurons. Depending on the pattern of disease onset, approximately equal numbers of patients have upper limb-,lower limb- or bulbar-onset (1).The cerebellum has traditionally been regarded as a crucial relay station for motor regulation, and has multiple reciprocal connections with the motor regions implicated in ALS (2).We previously demonstrated cerebellar atrophy in ALS, and the severity of atrophy in the inferior lobules and cerebellar vermis was found to correlate with measures of motor functional status (*Tan 2014, submitted*). However, the integrity of cerebellar neurons, particularly the large Purkinje cells (PC), which constitute the sole output neurons of the cerebellar cortex, has not been examined in ALS.

Objective: To assess PC in the lateral lobules and cerebellar vermis in ALS.

Methods: Following institutional approvals, paraffin-embedded tissue blocks from patients with pathologically-confirmed ALS (n = 30, 10 of each onset) and controls (n = 10) from the NSW Tissue Resource Centre were prepared for histological examination of neurons. The number of PC along two half lobules were quantified and normalized to umber of folia within each region-of-interest. ANOVA with posthoc Bonferoni compared PC densities/folia dichotomised by main clinical diagnosis or by type of onset.

Results: PC loss (~22%) was identified in the cerebellar vermis of ALS cases with lower-limb onset disease ($p < 0.05$) and correlated with disease duration (Rho = -0.38, $p = 0.04$).This was significant in comparison to upper limb- and bulbar-onset cases ($p = 0.001$),which did not differ from controls ($p > 0.05$). An average of ~10% loss of neurons occurred per year with no loss estimated at onset. No significant differences were identified with disease-onset in the lateral cerebellum.

Discussion: This is the first report of PC loss in the cerebellar vermis in ALS and we demonstrate a significant reduction in cases with lower limb-onset. The vermis is classically thought to receive somatic sensory input from ascending spinal pathways and be involved in the kinematics of ongoing movement. However, recent evidence in animal models have challenged this theory and shown that some PC located in the vermis receive dense direct input from motor neurons in the motor cortex and innervate lower but not upper limb motor neurons in the spinal cord (3, 4).Our findings suggest that such vermal PC are preferentially vulnerable in lower limb-onset ALS, most likely as a consequence of early distal axonopathy. This is likely to impact more widely on vermal function in lower-limb ALS patients.

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P194 MORPHOLOGICAL EVIDENCES OF GLIAL INFLAMMATION THAT CONFER SPINAL WHITE MATTER DEGENERATION OF SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

HAYASHI S[1], YAMASAKI R[2], MASAKI K[1], MURAI H[1], OKAMOTO K, KIRA J-I[1]

[1]*Department of Neurology,* [2]*Department of Neurological Therapeutics, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan,* [3]*Geriatrics Research Institute and Hospital, Maebashi, Japan*

Email address for correspondence: cho7pa3@yahoo.co.jp

Keywords: glia, inflammation, MCP1

Background: We previously reported that glial inflammation (GI) markers were elevated in the CSF of sporadic amyotrophic lateral sclerosis (1). However, morphological profiles regarding GI are poorly understood. In 70% of ALS cases infiltrations of massive microglia/macrophage (Mi/MΦ) with foamy appearance were observed not only in the cort

(CST), but also in the anterolateral funicles outside the CST (ALFoc), corresponding to the distribution of myelin pallor (2). Classical antibodies against CD 68 or Iba1, however, could not discriminate whether Mi/MΦ in the CNS were derived from peripheral monocytes or resident microglia. Regarding this issue, Yamasaki *et al.* demonstrated that chemokine receptor, CX3CR1, was expressed on resident microglia, while CCR2, on infiltrating monocytes (3). These findings might offer morphologically novel evidence of GI in sALS tissues.

Objectives: To characterize glial reactions and their related immune cells in sALS spinal white matter.

Methods: Ten-percent buffered formaline-fixed, paraffin-embedded 5-μm-thick transverse thoracic cord sections of sALS cases ($n = 7$) and non-ALS cases (controls, $n = 5$) were examined by immunohistochemical methods. The antibodies used were against: Iba-1 (Mi/MΦ); CD68 (Mi/MΦ); CD11c (dendritic cells); CCR2 (monocyte); CX3CR1 (resident microglia); MCP-1 (ligand of CCR2); fractalkine (ligand of CX3CR1); CD45RO (T cells); CD20 (B cells); iNOS; and GDNF. Degree and distribution of infiltrating cells in both CST and ALFoc were examined, and immunoreactive cells in each region were quantified for statistical comparison. For correlation analysis, data were obtained from 30 spinal cord sections of five sALS patients.

Results: Compared to controls, sALS spinal cords showed CD68-, Iba1-, CCR2-, and CD11c-immunopositive cells in the ALFoc as many as those in the CST ($p > 0.05$), while the numbers of T cells in the CST were significantly increased than those in the ALFoc ($p < 0.01$). Many MCP-1-immunopositive granules were detected not only in the entire (CST + ALFoc) anterolateral funicles (ALF), but also in the posterior funicles (PF). There were positive correlations between the numbers of MCP-1- vs. CD11c- ($r = 0.737$, $p < 0.001$), MCP-1- vs. CD68- ($r = 0.439$, $p < 0.05$), and MCP-1- vs. Iba1-immunopositive cells ($r = 0.450$, $p < 0.05$), encompassed in the entire ALF. There were intense iNOS-immunopositive foamy cells in the entire ALF, but scarce on GDNF-immunoreactivities. No pathological findings were observed regarding CX3CR1-, fractalkine-, and CD20-immunostainings in the CST, ALFoc, and PF of all specimens.

Discussion and conclusion: Mi/MΦ in the entire ALF of sALS spinal cords might originate from peripheral monocytes attracted by MCP-1, and have neurotoxic nature, suggesting that MCP1 could be one of the therapeutic targets to ameliorate white matter degenerations of this disorder.

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P195 CEREBRAL LESIONS OF AMYOTROPHIC LATERAL SCLEROSIS (ALS) PATIENTS IN COMMUNICATION ABILITY STAGE V: TOTALLY LOCKED-IN STATE

HAYASHI K[1], MOCHIZUKI Y[1,2], NAKAYAMA Y[3], NAGAO M[1], SHIMIZU T[1], KOMORI T[1], ARAI N[3], NAKANO I[1]

[1]*Tokyo Metropolitan Neurological Hospital, Tokyo, Japan,* [2]*Tokyo Metropolitan Kita Medical and Rehabilitation Center for the Disabled, Tokyo, Japan,* [3]*Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan*

Email address for correspondence: kentaruneurological@gmail.com

Keywords: totally locked-in state, communication neuropathological findings

Background: We determined stage classifications based on communication ability and analyzed the characteristics of ALS patients. About 13% of patients (1) with ALS on long-term mechanical ventilation (LTMV) develop into a totally locked-in state (2), where all voluntary movements have been lost and communication is impossible by any means (communication stage V (3)). Neuropathological features of stage V ALS patients include severe degeneration of motor neurons and other systems. However, findings of cerebral lesions have not been reported.

Objective: To determine the characteristics of cerebral lesions in stage V patients.

Method: In four autopsied patients with ALS at stage V, we investigated the radiological profiles at the beginning of stage V as well as pathological features.

Results: Means and ranges of disease onset, disease duration, duration from onset to LTMV, and onset to stage V were 40 years (13–57), 14.8 years (9–26), 18.5 months (8–24), and 6.5 years (3–10), respectively. Patient 1's symptoms were sporadic, and displayed an accumulation of transactivation response DNA-binding protein of 43 kDa (TDP-43). Patients 2 and 3 had copper/zinc superoxide dismutase (SOD1) gene mutations (p.V118L, p.C146R) respectively. Patient 4 had a p.P525L mutation in the fused in sarcoma (FUS) gene. They had no cognitive disturbances from onset to stage V or history of brain ischemia. Radiologically, all patients displayed mild frontal and temporal lobe atrophy at the beginning of stage V.

Neuropathological findings: All patients exhibited common elements including severe degeneration of both upper and lower motor neurons and severe fiber loss in the tegmentum of the brainstem and the anterolateral funiculus of the spinal cord. The globus pallidus and substantia nigra showed mild neuronal loss and gliosis. The patients also exhibited certain variations. Patient 1 displayed neuronal loss in the frontal and temporal cortex and severe fiber loss in the cerebral white matter. Patients 2 and 3 exhibited relatively preserved frontal/temporal lobes, but displayed severe fiber loss in the posterior column of the spinal cord. Patient 4 showed neuronal loss in the frontal cortex and fiber loss in the cerebral white matter.

Discussion and conclusion: All patients radiologically displayed mild frontal and temporal atrophy at the beginning of stage V. However, pathological findings in terms of cerebral lesion indicated variations. Degeneration of the cerebrum of ALS patients progresses even after stage V, while disease progression may be distinct in each patient.

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P196 RESPIRATORY-AIDED, LONG-TERM SURVIVAL CASES OF AMYOTROPHIC LATERAL SCLEROSIS (ALS) WITH COMMUNICATION ABILITIES, MOTONEURON SYSTEM-CONFINED DEGENERATION, AND SCANTY TDP-43 AGGREGATION-A SUBGROUP OF ALS?

MOCHIZUKI Y[1,2], HAYASHI K[1], NAKAYAMA Y[3], SHIMIZU T[1], KAMIDE M[4], OGINO M[5], KOMORI T[1], ISOZAKI E[1], NAKANO I[1]

[1]*Tokyo Metropolitan Neurological Hospital, Tokyo, Japan,* [2]*Tokyo Metropolitan Kita Medical and Rehabilitation Center for the Disabled, Tokyo, Japan,* [3]*Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan,* [4]*Atsugi City Hospital, Kanaagaa, Japan,* [5]*Kitasato University School of Medicine, Kanagwa, Japan*

Email address for correspondence: mochi@nihon-u.ne.jp

Keywords: totally locked-in state, long-term mechanical ventilation (LTMV), neuropathological findings

Background: About 13% of patients (1) with ALS surviving on long-term mechanical ventilation (LTMV) develop into a totally locked-in state (TLS) (2), which refers to a state in which all voluntary movements are lost and communication by any means is impossible. However, it is known that some respirator-aided, long-term survival patients maintain communication abilities. The position that the latter group occupies along the spectrum of ALS cases has not been described.

Objective: To clarify, along the ALS cases, the position of the long-surviving ALS subgroup with good communication abilities.

Methods: Three autopsied ALS cases with an approximately 30-year survival and good communication abilities were clinicopathologically investigated in direct comparison with previously reported ALS cases suffering from a TLS (2, 3).

Case reports: Clinical course: Age of onset and disease duration of cases 1, 2, and 3 were 48, 55, and 31, and 28, 29, and 33 years, respectively. Case 1 underwent a tracheostomy 8 years after disease onset, and required mechanical ventilation regularly 26 years after onset. Case 2 and case 3 underwent mechanical ventilation 14 and 6 years after disease onset, respectively. Case 1 and 3 were in a state of complete tetraplegia and case 2 was able to move only his knee joint a little, and the three cases were in a state of bulbar palsy. However they could communicate well via eye movements.

Neuropathological findings: In all the cases, both upper and lower motor neurons were markedly degenerated while the brainstem tegmentum was preserved. Multiple system degeneration, a characteristic pathology of ALS in the TLS (2), was not seen. A few normal-looking motor neurons remained in the anterior horn of the spinal cord. Neither hypertrophic astrocytes nor macrophages were observed. There were no TDP-43-immunoreactive inclusions in the lower motor neurons of any case and only occasional ones in the cerebral cortex of case 3. Bunina bodies were not seen.

Discussion and conclusion: Each of the present cases displayed a much slower disease progression into required respiratory assistance compared with typical ALS patients and also maintained good communication in spite of survival on LTMV. Neuropathological findings of remaining good-shaped motor neurons, motoneuron system-confined degeneration, and a few TDP-43-immunoreactive inclusions indicate a significant difference from findings of ALS in the TLS. Thus, our clinicopathological investigations of these three cases give rise to the possibility that there is a distinct subgroup characterized by the above-mentioned features.

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THEME 9 *IN VITRO* EXPERIMENTAL MODELS

P197 THE ACADEMIC MEDICAL CENTRE ALS BRAIN TISSUE BANK AND DOIS: A FEASIBILITY STUDY

CASULA M, TROOST D

Academic Medical Centre, Amsterdam, The Netherlands

Email address for correspondence: m.casula@amc.nl

Keywords: brain tissue bank, datacite, digital object identifiers (DOI's)

Tissue banks serve a useful purpose insofar as they host a valuable scientific resource but their full potential is often not realized because samples are not easily discovered. Even when tissue samples are made available, the work associated with their collection and curation is often not acknowledged and financial support for the maintenance of these important collections is often lacking as well. With the emergence of data citation infrastructures, notably DataCite, there is the potential to address these issues while still respecting sample confidentiality.

With the aim of encouraging discovery and acknowledgement of tissue samples for ALS research, a feasibility study was undertaken at the Neuropathology Department at the AMC in Amsterdam to assign DataCite DOIs (Digital Object Identifiers) to the tissue samples of the ALS Brain Tissue Bank. This tissue bank consists of a unique collection of brain and spinal cord tissue of ~220 ALS patients who donated their body to science for research on ALS. The feasibility study involved assigning DataCite DOIs to a subset of 50 spinal cord paraffin samples. Assigning DataCite DOIs is of benefit to both the custodian of the tissue samples and the end-user because the former is cited for their contribution and the latter benefits from access to a valuable resource. Thus, in the circumstance that the work is published, the contribution of all concerned is acknowledged, potentially also leading to further use of the tissue bank.

While the confidentiality of the samples is respected, there is always a limited amount of bibliographic information (metadata) about the associated data set that is in the public domain that allows the samples to be discovered. The result is that for the ALS Tissue Bank, approval is always required form the curator before research collaborations are initiated.

In conclusion, when used to identify individual tissue samples, DataCite DOIs have the potential to encourage a better use of the ALS Brain Tissue Bank and hence stimulate research of ALS pathogenesis.

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P198 DEVELOPMENT OF IN VITRO MODELS OF ALS

PUGH V[1], POWELL L[2], MATTHEWS T[2], RATTRAY M[3], SWEENEY J[2]

[1]*University of Reading, Reading, UK,* [2]*University of Huddersfield, Huddersfield, UK,* [3]*University of Bradford, Bradford, UK*

Email address for correspondence: v.pugh@bradford.ac.uk

Keywords: in vitro model, neuroprotection, excitotoxicity

Background: Riluzole is the licensed neuroprotective drug for the treatment of amyotrophic lateral sclerosis (ALS). Riluzole is proposed to have various effects at clinically relevant concentrations including inhibition of Na+ currents (1), potentiation of calcium-dependent K+ currents (2) and inhibition of glutamate release (3). Although riluzole is clinically proven to extend lifespan in ALS patients, it has limited efficacy, extending lifespan by three months, on average (4). In order to find drugs with an improved potency, robust assays must first be developed in which to test novel compounds.

Objectives: We have developed a range of neurotoxicity assays against which to test a variety of compounds, including novel riluzole derivatives. The assays have been designed to mimic particular features of the pathological process observed in motor neurone disease.

Methods: Primary cultures of mouse cortical neurones and motor neurones were exposed to kainate, NMDA, tunicamycin and arsenite to induce cell damage or neuronal death. FM1-43 and Fura 2 AM fluorescent dyes were used to observe any loss of synaptic activity and an increase in intracellular calcium, respectively, as read outs of excitotoxicity. Morphological assessment of these cells was also conducted using MAP2 immunocytochemistry.

Results: Under all treatment paradigms we were able to induce degradation of neuronal processes in both cortical and motor neurones in a concentration dependent manner. We have observed an increase in Fura 2 AM fluorescence and a reduction in FM 1-43 fluorescence following exposure to toxins with respect to vehicle treated controls.

Discussion and conclusion: Using these assays we found that the toxins we used are capable of inducing excitotoxicity and ultimately cell death. The reduction in FM 1-43 fluorescence suggests a loss of synaptic vesicle recycling under excitotoxic treatment and ultimately a loss in synaptic activity. The increase in Fura 2 AM staining with lower concentrations of kainate and NMDA highlights an initial increase in intracellular calcium, an early event in the excitotoxicity in motor neurones seen in ALS. Upon developing these robust assays we will screen novel riluzole derivatives in these assays to find those which are capable of protecting neurones more than riluzole.

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P199 *IN VITRO* HIGHLY DIFFERENTIATED PRIMARY MUSCLE FIBER: A MODEL RECAPITULATING MUSCLE DIFFERENTIATION IN SOD1^{G93A} MOUSE

VILMONT V[1], CADOT B[1], GOMES ER[1,2]

[1]*Myology Research Center, Sorbonne Universités, UPMC Université Paris, Paris, France,* [2]*Instituto di Medicina Molecular, Lisbon, Portugal*

Email address for correspondence: vilmont@myologygroup.net

Keywords: muscle, in-vitro differentiation, SOD1^{G93A}

Background: Amyotrophic lateral sclerosis (ALS) is a highly debilitating fatal disease in humans with life expectancy ranging from two to five years after diagnosis. This pathology is characterized by weakness, muscle atrophy and spasticity leading to death in patients mostly due to respiratory failure caused by impaired diaphragm contraction. Muscle atrophy is believed to be triggered by the loss of upper motor neurons (UMNs) in the cortex and of lower motor neurons (LMNs) in brainstem and spinal cord. Genetic defects in the zinc/copper superoxide dismutase (SOD1) gene, has been linked to familial and sporadic ALS. While this model has been extensively used to study loss of MNs and neuromuscular junctions, less attention has been given to the potential role of impaired muscle development.

Objective: To investigate the possible pathophysiological link between muscle development and neuromuscular junction loss in the SOD1^{G93A} mice.

Methods: Primary myoblasts were isolated 6 to 7 days after birth from transgenic mice expressing the G93A mutant form of human SOD1 and from WT littermates. Myoblasts were directed to differentiate to advanced stages. Features of high differentiation like triad formation, movement of nuclei to the periphery, fiber thickness as well as neuromuscular junction dynamics were assessed.

Results: Myoblasts from SOD1^{G93A} could be differentiated *in vitro* like the WT littermates. Assessment of differentiation features revealed a decrease in the number of fibers with peripheral nuclei, impaired triad formation and reduced fiber thickness. Moreover, staining with bungarotoxin showed a reduced number of AchR clusters as well as a decrease in their length.

Discussion and conclusion: These preliminary data secure this protocol as a robust model to study highly differentiated fibers recapitulating muscle development. Our findings from SOD1^{G93A} mice revealed that muscle development is impaired in fibers taken from young mice which are still asymptomatic supporting the hypothesis that muscle development could be an independent trigger of neuromuscular junction loss and muscle atrophy.

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P200 INVESTIGATING MISFOLDED SOD1 IN ALS USING PATIENT-DERIVED CELLS

FORSGREN E[1], LEHMANN M[1], AMAROSO MW[2], GARCIA-DIAZ A[2], SANDOE J[3], KISKINIS E[3], NORDSTRÖM U[1], ANDERSEN P[1], BRÄNNSTRÖM T[1], EGGAN K[3], WICHTERLE H[2], HENDERSON CE[2], MARKLUND S[1], GILTHORPE J[1]

[1]*Umeå University, Umeå, Sweden,* [2]*Columbia University, New York, USA,* [3]*Harvard University, Boston, USA*

Email address for correspondence: Elin.Forsgren@neuro.umu.se

Keywords: induced pluripotent stem cells (iPSCs), SOD1, disease model

Background: Mutations in the superoxide dismutase 1 (SOD1) gene were first found to be associated with ALS (1). SOD1 mutations have now been found in 12–23% of familial ALS cases and in up to 6% of all cases of ALS. The leading hypothesis as to how *SOD1* mutations lead to ALS is by the induction of conformational changes within the protein, accumulation of unfolded SOD1 and the subsequent formation of cytotoxic aggregates. Significantly, the presence of aggregates of unfolded SOD1 in both familial and sporadic ALS suggests that unfolded SOD1 might represent part of a final common pathway leading to motor neuron death is ALS (2).

Numerous studies have alluded to different, potential mechanisms of unfolded SOD1 toxicity. However, the precise nature of the toxic SOD1 species is not yet clear. The pathogenesis of ALS is proposed to be due to both cell autonomous mechanisms acting in motor neurons, and non-cell autonomous ones, where other cells types such as astrocytes are involved (3). We have focused on the use of induced pluripotent stem (iPS) cells as a model system to investigate both cell and non-cell autonomous mechanisms of unfolded SOD1 toxicity *in vitro*, using patient-derived cells.

Methods: iPS-derived motor neurons and astrocytes have been generated from different ALS patients carrying SOD1 mutations, as well from non-diseased controls. The amounts of unfolded SOD1 in patient derived fibroblasts, iPS cells, iPS-motor neurons and astrocytes were analysed.

Results: Our results show that: misfolded SOD1 is present in patient-derived cell cultures; amounts of misfolded SOD1 vary between cell types and SOD1 mutations; high amounts of misfolded SOD1 in neurons carrying G85S-SOD1 but even more in iPS-astrocytes; iPSCs are a useful system to study misfolded SOD1 in ALS; the presence of high levels of misfolded SOD1 could explain the selective vulnerability of motor area cells in ALS

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P201 THE EFFECTS OF METABOLIC DISTURBANCES ON THE LEVELS OF MISFOLDED SOD1 IN ALS PATIENT-DERIVED FIBROBLAST LINES

KESKIN I[1], FORSGREN E[1], GILTHORPE J[1], TOKUDA E[2], WEBER M[3], BIRVE A[1], ANDERSEN PM[1], MARKLUND SL[2]

[1]*Department of Pharmacology and Clinical Neuroscience,* [2]*Department of Medical Biosciences, Umea University, Umeå, Sweden,* [3]*Neuromuscular Diseases Unit/ALS Clinic, Kantonsspital St. Gallen, St. Gallen, Switzerland*

Email address for correspondence: isil.keskin@neuro.umu.se

Keywords: SOD1, misfolded, patient-derived fibroblast lines

Background: The neurotoxicity of mutant SOD1 is believed to be exerted by misfolded SOD1 species. The patho-mechanism is poorly understood but has been suggested to involve perturbation of mitochondria, induction of ER-stress, reduction of proteasome and autophagy efficiency and aggregation. Another unresolved feature of ALS is why carriers of *SOD1* mutations are apparently healthy until middle age, and then undergo rapid neurological decline. Perhaps age-related decline in proteostasis and energy metabolism contributes, amplified by vicious circles that increase the levels of misfolded SOD1 in the tissue.

Objectives: We have generated fibroblast lines from skin biopsies derived from 8 ALS patients carrying mutations in *SOD1* (A4V, H46R, E78_R79insSI, N86S, D90A, L117V, D126fsX24, G127ins*tggg*); from 1 ALS and 1 FTD patient with *C9ORF72* GGGGCC-hexanucleotide repeat expansion, from 2 healthy control subjects. These cells, which express the mutant SOD1 under the native promoter, offer opportunities for exploration which are not accessible in other model systems. We used the cells to gain information on the effects of various metabolic disturbances on the levels of misfolded SOD1.

Methods: We cultured the fibroblast cell lines under a variety of stress conditions and with/without inhibitors. Misfolded SOD1 from fibroblast extracts was measured with a specific ELISA (misELISA) as described (1).

Results: All fibroblast lines derived from the ALS patients contained more misfolded SOD1 than those from control individuals. The proteasome inhibitor bortezomib caused marked increases in misfolded SOD1 levels. Induction of ER stress with tunicamycin, inhibition of mitochondria with rotenone, and suppression of autophagy with 3-methyladenine, also decreased the levels of misfolded SOD1. Hyperoxia might conceivably stabilize SOD1 by artificially promoting the C57-C146 disulfide bond. There were, however, no differences between cells cultured in physiological oxygen tension (4% O_2) and hyperoxia (20% O_2). Under none of the conditions could any detergent-resistant aggregates be demonstrated in the cell extracts.

Conclusion: Proteasome inhibition caused large increases in misfolded SOD1 levels. Other cellular perturbations occurring in ALS and aging did not per se induce increases in misfolded SOD1 levels.

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P202 NEUROPROTECTIVE POTENTIAL OF HUMAN MESENCHYMAL STROMAL CELLS IN ALS IN VITRO MODELS

BÖSELT S[1], RATH KJ[1], KLÖSS S[2], ESSER R[2], KÖHL U[2], PETRI S[1]

[1]*Department of Neurology,* [2]*Institute of Cellular Therapeutics, Hannover Medical School, Hannover, Germany*

Email address for correspondence: petri.susanne@mh-hannover.de

Keywords: cell therapy, mesenchymal stromal cells (MSC), apoptosis

Background: Mesenchymal stromal cells (MSC) are currently discussed as potential candidates for the therapy of neurodegenerative diseases such as ALS and are already investigated in clinical trials, mainly based on the assumption that non-neuronal cells can provide a more protective environment for degenerating motor neurons. We have previously shown protective effects of an MSC cell line *in vitro*, based on modulations of cellular expression of growth factors, cytokines and chemokines in neurons and non-neuronal cells (1).

Objectives: We intended to establish good manufacturing practice (GMP) isolation of human MSC from bone marrow available from healthy donors and to develop appropriate quality control. Protective effects and underlying mechanisms of action were studied in cellular models of ALS.

Methods: 10-colour flow cytometric protocols for quality control including detection of residual T and B cells were established and validated. Expression of MSC surface markers was studied by FACS analysis and laser-induced fluorescence microscopy. Differentiation potential into osteocytes, chondrocytes and lipocytes was confirmed. Mouse embryonic primary motor neurons (derived from both non-transgenic and mutant $SOD1^{G93A}$ transgenic mice), NSC-34 cells and glial cells (astrocytes) (derived again from both non-transgenic and mutant $SOD1^{G93A}$ ALS transgenic mice) were co-cultured with human MSC and the effects against staurosporine- and H_2O_2-induced cell death were determined by immunocytochemistry and MTT assay.

Results: Conditioned medium (MSC CM) as well as co-culture with MSC attenuated staurosporine - and H_2O_2-induced cell death in a concentration-dependent manner in primary motor neurons, NSC-34 cells and astrocytes. MSC-co-culture decreased neuronal mRNA expression of glutamate receptor 2 and of matrix metalloproteinase 9 (MMP-9). Ongoing analyses comprise MSC-mediated changes in gene- and protein expression of pro-and antiinflammatory mediators, neurotransmitters and corresponding receptors and growth factors.

Discussion and conclusion: Our data demonstrate that MSC have neuroprotective capacities, mediated by effects on apoptosis, oxidative stress and possibly neurotransmission and ER stress. Analysis of MSC-induced changes in motor neurons and glial cells, also regarding genotype and possibly disease-specific alterations can further clarify their mechanisms of action and therapeutic potential.

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P203 INVESTIGATING OLIGODENDROCYTE DYSFUNCTION IN ALS USING HUMAN STEM CELLS

MAGNANI D[1], LIVESEY MR.[1], BURR K[1], SELVARAJ BT[1], VASISTHA N[1], EVANS E[1], RAJAN R[1], STORY D[1], ZHAO C[1], BOROOAH S[1], SHAW C[3], VALLIER L[2], WYLLIE DJA[1], CHANDRAN S[1]

[1]*Euan Macdonald Centre, University of Edinburgh, UK,* [2]*Cambridge stem cell institute, Cambridge, UK,* [3]*UCL, London, UK*

Email address for correspondence: D.Magnani@ed.ac.uk

Keywords: oligodendrocytes, induced pluripotent stem cells (iPSCs), TDP-43

Background: In ALS/MND nerve cells degenerate leading to muscle weakness and paralysis. Another key cell in the brain and spinal cord is the oligodendrocyte (OL). These cells provide metabolic support and insulation to neurons and until recently were not believed to be involved in MND. However, studies of ALS human post mortem samples and the $SOD1^{G93A}$ mouse model of ALS, implicate OLs in the aetiopathogenesis of motor neuronal degeneration. Our aim is to investigate the cell autonomous defects of oligodendrocytes in ALS.

Methods: We have optimized a platform to efficiently convert human induced pluripotent stem cells (iPSCs) obtained from control and familial ALS patients, carrying the $TDP43^{M337V}$ and $TDP43^{G298S}$ mutation, into functional OL progenitors (OPCs) and OLs. Spinal cord patterned iPSC derived OPCs (OLIG2 + and PDGFRa+) can be expanded for several weeks in the presence of FGF and PDGFa. OPCs are further differentiated into OLs by removal of FGF and PDGFa.

Results: Upon differentiation very little overlap is observed (< 15%) between PDGFRa+ OPCs and O4 + OLs, while differentiated OLs begin to express myelin basic protein (MBP) (> 90%), becoming mature OLs within 10 days of differentiation. OL differentiation and maturation coincided with an increase in cell size and OL development was associated with an increase in the number and length of OL processes, as quantified by Sholl analysis. Furthermore, using pharmacological and biophysical approaches we have examined the profile of passive membrane properties of hPSC-derived OPCs through to their maturation into OLs. OL differentiation was associated with a loss of spiking activity and a reduced rectification of outward currents that indicated a change in the ion channel expression profile of OLs compared to those expressed in OPCs. Specifically, we observed decreases in tetrodotoxin-senstive voltage-gated Na^+-channels (OPCs: 29.1 ± 1.1 pA/pF, n = 18; OLs: 0 pA/pF, n = 5), TEA-sensitive delayed-outwardly rectifying K^+-channels (OPCs: 26.4 ± 0.4 pA/pF, n = 15; OLs: 0.5 ± 0.1 pA/pF, n = 9) and transient A-type K^+-channels (OPCs: 105.6 ± 4.5 pA/pF, n = 12; OLs: 8.1 ± 0.8 pA/pF, n = 6). Conversely, immunohistochemistry revealed prominent expression of inwardly-rectifying K^+-channel 4.1 subunits in OLs, whereas expression of this channel subunit was only negligibly detected in OPCs. This recapitulates ion channel expression profiles exhibited by native (rodent) OL-lineage.

Discussion and conclusion: Studying molecular and physiological differences between WT and mutant TDP oligodendrocyte lines is ongoing. Our work has established a platform to investigate cellular autonomy in MND with a focus on oligodendrocytes.

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P204 CHARACTERISATION OF TDP-43 DYSREGULATION IN C9ORF72 IPSC-DERIVED MOTOR NEURONS

SCABER J[1], MUTIHAC R[1], COWLEY S[2], TURNER M[1], TALBOT K[1]

[1]*Nuffield Department of Clinical Neurosciences, John Radcliffe Hospital,* [2]*Oxford Stem Cell Institute, Sir William Dunn School of Pathology, University of Oxford, Oxford, UK*

Email address for correspondence: jakub.scaber@ndcn.ox.ac.uk

Keywords: C9ORF72, induced pluripotent stem cells (iPSCs), TDP-43

Background: An expanded hexanucleotide (GGGGCC) 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). At post-mortem, patients with the hexanucleotide repeat have the typical TDP-43 positive inclusions that are detected in over 90% of patients with ALS, but also have TDP-43 negative inclusions that contain repeat-associated non-ATG (RAN) dipeptides translated from the intronic repeats. The role of TDP-43 pathology in C9ORF72 disease remains unknown.

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 at least 4 weeks before assessment of TDP-43 pathology.

Results: We detected significantly elevated levels of full-length TDP-43 protein in C9ORF72 iPSC-derived motor neurons, as well as a 35 kDa isoform. At the mRNA level, quantitative analysis of TDP-43 transcripts showed that levels of TARDBP mRNA were similar in both control iPSC-derived motor neurons and C9ORF72 iPSC-derived motor neurons. Oxidative stress induced by sodium arsenite treatment resulted in a marked decrease of soluble TDP-43 in C9ORF72 motor neurons compared to healthy motor neurons. Analysis of TDP-43 subcellular localisation was performed in both stressed and unstressed conditions. In the absence of oxidative stress, no differences were detected in TDP-43 localization between C9ORF72 and healthy iPSC-derived motor neurons (p values to follow).

Discussion and conclusion: In our iPSC-derived neuronal culture enriched for motor neurons, there is evidence of derangement of TDP-43 at the protein level but not at the mRNA level. Given that TARDBP mRNA is tightly regulated by TDP-43, this may suggest a disturbance in the autoregulatory mechanism of TDP-43.

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P205 G4C2 REPEAT-INDUCED TRANSLATIONAL REPRESSION IN A CELLULAR MODEL OF C9ORF72 ALS

ROSSI S[1,2], SERRANO A[1,2], GIORGI A[5], GERBINO V[3], NENCINI M[3], DI FRANCESCO L[5], SCHININÀ ME[5], CESTRA G[6], ACHSEL T[4], CARRÌ MT[2,3], COZZOLINO M[1]

[1]*Institute of Translational Pharmacology, CNR, Rome, Italy,* [2]*Department of Biology, University of Rome Tor Vergata, Rome, Italy,* [3]*Fondazione Santa Lucia, Rome, Italy,* [4]*Center for the Biology of Diseases, KULeuven, Leuven, Belgium,* [5]*Dipartimento di Scienze Biochimiche A. Rossi Fanelli, Università Sapienza, Rome, Italy,* [6]*Istituto di Biologia e Patologia Molecolari, CNR, Rome, Italy*

Email address for correspondence: mauro.cozzolino@ift.cnr.it

Keywords: C9ORF72, protein translation, Pur-alpha

Background: A common feature of non-coding repeat expansion disorders is the accumulation of RNA repeats as RNA foci in the nucleus and/or cytoplasm of affected cells. By sequestering BRNA binding proteins, these RNA foci can be toxic. The reduced effective concentration of these factors can then affect various steps of post-transcriptional gene regulation, such as alternative mRNA splicing, translational regulation, mRNA transport, or mRNA stability. However, the precise step that is affected by *C9ORF72* repeat expansions, the major genetic cause of amyotrophic lateral sclerosis, is still ill defined.

Objectives: We investigated the possible mechanisms whereby *C9ORF72* repeats might affect cell viability by identifying proteins that bind to *C9ORF72* repeats, and analysing their expression and subcellular distribution.

Methods: A 31 pure GGGGCC repeat $(G4C2)_{31}$ was obtained by *in vitro* ligation of complementary nucleotides, and cloned into mammalian expression plasmids. Mouse motoneuronal NSC34 cells and human HeLa cells transfected with $(G4C2)_{31}$ repeats were used as cellular models. Immunofluorescence analysis, coupled to fluorescence *in situ* hybridisation (FISH), was used to assess RNA foci formation and localization of binding partners. Global protein synthesis was monitored with the SUnSET method based on puromycin incorporation.

Results: To get insights into the mechanisms whereby *C9ORF72* might induce cell toxicity, we used an *in vitro*-transcribed biotinylated RNA containing the $(G4C2)_{31}$ repeats to identify binding proteins. Through mass spec analysis of bands excised from SDS PAGE, we were able to identify many different factors involved in post-transcriptional gene regulation. In particular, members of the hnRNP and SR family of proteins, both regulators of alternative splicing, as well as translational regulators, including initiation and elongation factors, Pur-alpha, Pur-beta and other translation regulatory proteins were also found. The expression of $(G4C2)_{31}$ repeats is sufficient to induce the formation of intra-nuclear RNA foci in NSC34 and HeLa cells. A significant, although not complete, sequestration of some of the above factors into RNA foci was observed. Most strikingly, $(G4C2)_{31}$ repeat widely affects the overall distribution of Pur-alpha and its binding partner FMRP, which accumulate into intra-cytosolic granules that are positive for the expression of stress granules markers. In these conditions, global protein synthesis turned out to be decreased, as measured by the reduction in puromycin incorporation into nascent peptide chains.

Discussion and conclusion: Our observations show that *C9ORF72* repeats are able to activate a stress response that leads to a general reduction of translation, and suggest that this might be due to *C9ORF72* ability to bind and sequester translational regulators.

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P206 GENERATION INTEGRATION-FREE AND XENO-FREE INDUCED PLURIPOTENT STEM CELLS FROM BLOOD CELLS OF FALS PATIENT CARRYING FUS GENE MUTATION

LIU X[1], GAO S[2], YU C[2], DENG M[1]

[1]*Peking University Third Hospital, Beijing, China,* [2]*Tongji University, Shanghai, China*

Email address for correspondence: dengmin1706@gmail.com

Keywords: induced pluripotent stem cells (iPSCs), FUS gene mutation, motor neuron

Objective: To generate integration-free and Xeno-free iPSCs from ALS patients carrying FUS gene mutation by introducing episomal plasmids into peripheral blood mononuclear cells with nucleofection, and to provide safe and reliable resources for clinical application in the future. In addition, we aimed to differentiate ALS-iPS into motor neurons and provide cell-based disease model for ALS disease in Han Chinese.

Methods: Transfer non-integrated episomal plasmid into peripheral blood mononuclear cells from FALS with FUS P525L gene mutation by using Amaxa Nucleofector® I and other related nucleofection reagents. Remove the effect of animal-derived feeder cells and other components on human iPS cells by using E8 culture medium. Karyotype analysis, immunofluorescence staining, quantitative RT-PCR, teratome formation assay and bisulfate genomic sequencing were used to identify the pluripotency of these iPS cell lines. In addition, motor neurons were derived from these iPS cells by inhibiting SMAD pathway.

Results: 6 integration-free iPS cell lines were established from ALS patients carrying the P525L mutation. Cell lines can be stably cultured in Xeno-free medium longterm, demonstrate pluripotency and are similar to human ES cells. Furthermore, motor neurons were successfully induced from these iPS cells. Dislocation and aggregates of mutant FUS protein were observed not only in motor neurons but also in iPS cells.

Discussion and conclusion: Gene mutations do not affect the reprogramming of blood cells and pluripotency of iPS cells, nor does it prevent differentiation of motor neurons. The non-integrated ALS-specific iPS cell lines we generated can avoid the integration of foreign genes into the genome, and remove the impact of animal-derived feeder cells and other components on human iPS cells, which is more suitable for clinical application in the future. Furthermore, the disease model we generated can recapitulate key aspects of ALS pathogenesis. They offer a cell-based disease model and indispensible resource for further elucidating ALS disease pathogenesis and screening appropriate drug candidates in Han Chinese.

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P207 NOT AS FUS-SY AS PREVIOUSLY THOUGHT; A STUDY OF THE CHANGING SUB-CELLULAR LOCALISATION OF TDP-43 AND FUS IN RESPONSE TO CELL STRESS

JAMES J, MOUJALLED D, DUNCAN C, LIDDELL J, WHITE A

The University of Melbourne, Parkville, Victoria, Australia

Email address for correspondence: jjames@student.unimelb.edu.au

Keywords: FUS, TDP-43, stress-granule

Background: Mutations in the RNA binding proteins TDP-43 and FUS have been associated with familial and sporadic forms of MND. Recent findings have implicated RNA binding proteins such as TDP-43 in stress response and have shown that both wild-type and mutant TDP-43 incorporate into stress granules. However, it is widely believed that wild-type FUS does not incorporate into stress granules (1), thus differing from TDP-43.

Objectives: To investigate changes in sub-cellular localisation of TDP-43, and FUS in response to various inducers of stress in cell culture; and to determine if wild-type FUS incorporates into stress granules.

Methods: Primary cortical neurons, and SH-SY5Y neuroblastoma cells, were grown in culture and treated with various inducers of sub-toxic cell stressors which are known to cause mitochondrial impairment or oxidative stress; sodium arsenite, paraquat, ammonium ferric (III) citrate ($n \geq 3$). The level of cellular LDH release was measured to indicate cell death. Changes in cellular metabolic activity were measured using both the MTT assay and the bioluminescent ATP assay. Cells were fixed and immunocytochemistry was used to determine TDP-43 and FUS localisation, and incorporation into stress granules, using the markers TIAR and HuR. The software Imaris v7.1 was used on confocal images to determine changes in nuclear vs cytosolic distribution of proteins.

Results: Wild type FUS does incorporate into stress granules with 300μM sodium arsenite treatment for 3hr in culture, but not 1hr in culture. A significant number of stress granules were induced ($n \geq 3$, $p < 0.05$ increase of 36.7 granules per 100 cells). FUS translocated from the nucleus to the cytosol with this treatment ($n \geq 3$, $p = 0.0432$). These changes occurred with minimal cell death (no significant LDH release, $p = 0.696$) but a significant decrease in metabolic activity was observed by reduction in MTT metabolism ($n \geq 6$, $p < 0.0001$), and confirmed via the ATP assay ($n \geq 3$, $p = 0.0171$).

Discussion and conclusion: It has become widely accepted that wild type FUS (1) does not incorporate into stress granules, however this study shows that during cell stress (ATP depletion in response to sodium arsenite treatment), wild-type FUS moves from the nucleus to the cytosol and incorporates into stress granules, yet this occurs several hours after TDP-43 incorporates into stress granules. This suggests different roles for TDP-43 and FUS in response to cell stress.

Conclusion: Wild type FUS incorporates into stress granules with prolonged stress treatments. This result suggests that the normal role of FUS during conditions of metabolic impairment is to translocate from the nucleus to the cytosol and to regulate cytosolic RNA processing.

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P208 PUR-ALPHA INHIBITS MRNA TRANSLATION AND AFFECTS STRESS GRANULES MODULATING LOCALIZATION AND ACTIVITY OF FUS

DI SALVIO M[1,2], GERBINO V[3], PICCINNI V[1,2], CAMERINI S[4], CHELLINI L[5], CARRÌ MT[3,5], LORENI F[5], COZZOLINO M[3,6], CESTRA G[1,2]

[1]*IBPM, Istituto di Biologia e Patologia Molecolari, CNR, Rome, Italy,* [2]*University of Rome "Sapienza", Dept. of Biology and Biotechnology Charles Darwin, Rome, Italy,* [3]*Laboratory of Neurochemistry, Fondazione Santa Lucia, IRCCS, Rome, Italy,* [4]*Dept of Cell Biology and Neurosciences, Istituto Superiore di Sanità, Rome, Italy,* [5]*Department of Biology, University of Rome "Tor Vergata", Rome, Italy,* [6]*IFT, Istituto di Farmacologia Traslazionale, CNR, Rome, Italy*

Email address for correspondence: gianluca.cestra@uniroma1.it

Keywords: Drosophila, retinal degeneration, Pur-alpha

Background: FUS protein carrying ALS causative mutations mislocalizes and coalesces in stress granules (SGs). We identified Pur-alpha as a protein that specifically binds to mutated FUS. Pur-alpha is a highly conserved protein that interacts, in a sequence-specific fashion, with single-stranded DNA and RNA (1). Interestingly, Pur-alpha was recently demonstrated to bind to GGGGCC expanded repeats of C9ORF72 gene, one of the most frequent mutations associated with ALS (2). In a *Drosophila* model of neurodegeneration caused by GGGGCC repeats expression, Pur-alpha ameliorates the phenotype (3).

Objectives: We investigated the protein-protein interaction network around the C-terminus of FUS and we identified Pur-alpha as a FUS binding protein. Thus we addressed the role of Pur-alpha in the regulation of stress granules and its effect on localization and toxicity of FUS.

Methods: We performed affinity purification from rat brain extracts and mass spectrometry to identify FUS interacting proteins. We verified FUS/Pur-alpha interaction in pull down and co-immunoprecipitation experiments. We addressed the role of Pur-alpha in the regulation of stress granules in mammalian cell lines and its role in FUS-mediated neurodegeneration in *Drosophila melanogaster* transgenic flies.

Results: We have found that Pur-alpha binds specifically to the C-terminal region of mutant FUS in an RNA-dependent fashion and that the two proteins colocalize in SGs. We also report that Pur-alpha associates with monomeric ribosomes and polyribosomes. Interestingly, phosphorylation of the alpha subunit of eIF2 initiation factor is upregulated by either Pur-alpha or mutated FUS overexpression. According to an inhibitory effect of Pur-alpha on mRNA translation, its expression suppresses SG coalescence and relocalizes mutated FUS in a cytosolic diffuse pool. RNAi-mediated ablation of Pur-alpha produces locomotion defects in *Drosophila* indicating a pivotal role for this protein in motoneuronal function. Surprisingly, the ectopic expression of Pur-alpha in different *Drosophila* tissues, although is sufficient to produce a diffused pool of mutated FUS, exacerbates neurodegeneration.

Discussion and conclusion: We propose that mutated FUS and Pur-alpha affect parallel pathways converging on the inhibition of protein translation, and that translation inhibition is involved in FUS-mediated ALS. Since Pur-alpha expression in *Drosophila* motoneurons, as well as in cultured cells, reduces mutant FUS aggregation and worsens the degenerative phenotype, we propose that soluble mutant FUS in the cytoplasm is more dangerous for the cell than clustered in SGs.

In conclusion, we unveil a specific physical and genetic interaction between Pur-alpha and FUS and a novel functional role of Pur-alpha in the regulation of SGs and protein synthesis, which affects localization and toxicity of mutant FUS proteins.

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P209 MUTANT FUS/TLS EXPRESSION INHIBITS PML NUCLEAR BODY TURNOVER

KO HK, ZHOU H, HAYWARD L

Univ of Massachusetts Medical School, Worcester, MA, USA

Email address for correspondence: lawrence.hayward@umassmed.edu

Keywords: FUS, PML, nuclear body

Background: More than 30 dominant mutations in the nucleic acid binding protein FUS/TLS (fused in sarcoma/translocated in liposarcoma) cause ~5% of familial ALS cases. Multiple roles of FUS/TLS have been proposed in both the nucleus and the cytoplasm, but the mechanism(s) by which mutant FUS/TLS renders motor neurons vulnerable to degeneration in ALS have not been specifically defined. The promyelocytic leukemia (PML) protein is a major constituent of PML nuclear bodies (PML-NBs), which are dynamic structures implicated in nuclear homeostasis and stress responses that may influence motor neuron health.

Objectives: Because the functional roles of PML-NBs and associated components remain incompletely understood, we sought to test whether expression of human WT or mutant FUS/TLS variants may perturb the dynamics of PML-NB formation, localization, composition, or turnover.

Methods: We expressed human GFP-FUS/TLS variants (wild type or R495X, R521G, or P525L mutants) stably in HEK293 cells and performed immunostaining for GFP, PML, proteasome 11S subunit, and the PML-associated protein Daxx. Cells were exposed to stress for 1–24 h using arsenic trioxide (ATO), an agent known to induce PML-NBs acutely and to potentiate subsequent PML protein degradation by the proteasome.

Results: We observed that exposure to 1 μM arsenic trioxide (ATO) acutely induced the formation of large PML-NBs within 1–2h for both mutant and control HEK293 cells. After 12–24h of exposure to ATO, cells expressing wild type FUS/TLS showed a reduction in PML-NBs, as would be expected due to normal turnover of PML bodies by the proteasome. In cells expressing mutant FUS/TLS, however, large PML-NBs persisted and localized to perinucleolar regions. Increased PML protein accumulation was detected in the RIPA-insoluble fraction from cells expressing FUS/TLS mutants compared to control. Within 1h of ATO exposure, the 11S proteasome subunit colocalized with PML-NBs in both mutant and control cells, and this colocalization persisted in the large PML-NBs of mutant cells at 24h. Immunostaining for the PML-associated protein Daxx, which has been implicated in stress-induced cell death and a Fas-induced pathway relevant to motor neuron degeneration, revealed strong accumulation of Daxx in large PML-NBs of cells expressing mutant FUS/TLS.

Discussion and conclusion: These results suggest that mutant FUS/TLS expression in HEK293 cells inhibits the normal turnover of PML-NBs during prolonged stress despite effective recruitment of the 11S proteasome subunit to PML-NBs. Ongoing studies are directed toward understanding the mechanism of failure to degrade PML-NBs and determining whether the accumulation of PML-NBs, or associated proteins such as Daxx, may impair cell viability.

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P210 INVESTIGATING THE ROLE OF THE NBAF COMPLEX IN ALS

TIBSHIRANI M, DURHAM H

McGill University, Montreal, Quebec, Canada

Email address for correspondence: michael.tibshirani@mail.mcgill.ca

Keywords: chromatin remodeling, dendrites, histone acetylation

Background: The nBAF (neuronal Brg1-associated factors) complex is a chromatin remodeling complex important for neuronal differentiation, dendritic arborization and synaptic plasticity. Complexes include multiple BAF subunits, CREST and Brg1 (1). Dendritic attrition and cytoplasmic accumulation of the DNA/RNA binding proteins FUS and TDP-43 are associated with ALS (2, 3). In addition, FUS interacts with components of the nBAF complex, including Brg1 (4). Recently, CREST variants were discovered in ALS trios, suggesting a role for the nBAF complex in ALS (4).

Objective: In the context of identifying epigenetic changes in motor neurons resulting from altered nuclear/cytoplasmic distribution of wild type and mutant FUS/TDP-43 and their binding partners, we examined components of the nBAF

complex and the relationship of their distribution to maintaining dendritic architecture.

Methods: Spinal cord-DRG cultures were prepared from E13 mouse embryos. Motor neurons in mature cultures were microinjected with plasmids encoding flag-tagged WT or mutant FUS or TDP-43 along with mCherry to visualize cell morphology. Dendritic architecture was quantified using the MATLAB script Bonfire (Firestein Lab).

Results: In motor neurons expressing mutant FUS or TDP-43, dendritic branching and length were decreased and terminal dendritic branches were lost compared to WT or empty vector controls. Furthermore, Brg1 was depleted from the nucleus in neurons with redistribution of WT or mutant proteins to the cytoplasm. Overexpression of Brg1 was sufficient to completely prevent the dendritic attrition caused by mutant FUS ($p = 0.00055$) or TDP-43 ($p = 1.98e\text{-}6$). Treatment with the Brg1 inhibitor, PFI-3, dramatically reduced dendritic arborization in neurons, replicating the effect of mutant FUS/TDP-43. Brg1 contains bromodomains, which have a high affinity for acetylated lysine in histones; treatment with the histone deacetylase inhibitor Vorinostat, preserved actetylation of H3K9/14 in motor neurons expressing mutant FUS or TDP-43 and prevented the nuclear depletion of Brg1 and dendritic attrition associated with these mutant proteins.

Discussion and conclusion: Attrition of upper and lower motor neuron dendrites is a prominent feature in ALS, which would contribute substantially to dysfunction (4). Our study links the cytoplasmic accumulation of FUS and TDP-43, which occurs in familial and sporadic forms of ALS, to epigenetic changes including disruption of nBAF complex activity, leading to retraction of the dendritic arbor. Brg1 appears to be a master regulator, and potential therapeutic target, since maintaining Brg1 in the nucleus was sufficient to prevent dendritic attrition, despite redistribution of FUS andTDP-43 to the cytoplasm. The influence of other nBAF components is being investigated.

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P211 GRASPS™: A NOVEL, SIMPLE AND ROBUST METHODOLOGY TO IDENTIFY ALS-ALTERED MRNA MOLECULES UNDERGOING PROTEIN SYNTHESIS

DODD JE, WALSH MJ, SHAW PJ, HAUTBERGUE GM

SITraN, University of Sheffield, Sheffield, UK

Email address for correspondence: g.hautbergue@sheffield.ac.uk

Keywords: RNA dysregulation, translating mRNA molecules, pathophysiology

Background: Dysregulation of messenger RNA (mRNA) metabolism constitutes a major determinant in the pathogenesis of several ALS-subtypes (1, 2). Many research groups around the world have characterized hundreds to thousands of abnormal RNA-processing events in cell and animal models of ALS as well as in post-mortem CNS tissues from patients. However, these studies (i) did not allow discrimination of pathophysiological events from those which were consequent upon initial dysregulations, and (ii) did not investigate at genome-wide level whether altered mRNAs are translated into proteins.

Objectives: Since proteins are ultimately involved in cell fate, survival or neurodegeneration, we set out to develop a reliable method to identify the levels and sequences of actively translated mRNA molecules, in order to apply this methodology to TDP-43 ALS-inducible cell models that present widespread dysregulation of RNA metabolism.

Methods: GRASPS™ is based on stalling protein synthesis using potent translation inhibitors such as cycloheximide (3) or the bacterial toxin BLF1 (4), as well as various cross-linking reagents. Once stalled the stable translation complexes are purified under stringent conditions that also dissociate peripheral ribosomal components and contaminating RNA-binding factors indirectly bound to ribosomes. The specific purification of stalled ribosomes enables the trapped translating mRNA molecules to be purified and then identified using next generation RNA sequencing.

Results: We have established proof-of-principle for this methodology and named it GRASPS for Genome-wide RNA Analysis of Stalled Protein Synthesis. High purity and integrity of isolated ribosomes and associated-mRNA molecules were validated using mass spectrometry and the Bioanalyser (*Agilent*). The method was also functionally validated using quantitative RT-PCR analysis of a Green Fluorescent Protein (GFP) mRNA reporter in GRASPS™ fractions from stable GFP-inducible human HEK cell lines with or without a mutation of the initiating start codon. We next applied the novel methodology to the investigation of the functional consequences of RNA dysregulation in TDP-43 Q331K ALS-inducible non-neuronal (HEK) and motor-neuron-like (NSC-34) cell models. We are currently proceeding with the Next Generation RNA sequencing analysis.

Discussion and conclusion: We have developed a novel method, GRASPS™, which allows isolation and identification of translating mRNA molecules at genome-wide levels. We will be identifying the first ALS translatomes in TDP-43 Q331K cell models. In addition, GRASPS™ is not only directly transferable to other ALS subtypes and for use in tissues, but also to any other disease in which RNA dysregulation may be involved.

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P212 CHARACTERIZATION OF MATRIN 3, A NEW RNA BINDING PROTEIN LINKED TO ALS

BOEHRINGER A[1], PIORO E[2], CHIÒ A[3], TRAYNOR BJ[4], BOWSER R[1]

[1]*Barrow Neurological Institute, Phoenix, AZ, USA,* [2]*Department of Neurology Cleveland Clinic, Cleveland, OH, USA,* [3]*Department of Neuroscience University of Turin, Turin, Italy,* [4]*Laboratory of Neurogenetics, National Institutes of Health, Bethesda, MD, USA*

Email address for correspondence: aboehrin@asu.edu

Keywords: Matrin 3, TDP-43, proteomics

Background: We recently described four mutations in the *MATR3* gene encoding the nuclear matrix protein Matrin 3 that are associated with ALS in four families around the world (1). Matrin 3 is a RNA binding protein and by immunohistochemistry is located to the nucleus of motor neurons and glia in the spinal cord. While cytoplasmic inclusions containing Matrin 3 are rare, we detect increased Matrin 3 immunoreactivity in motor neuron nuclei in ALS subjects and diffuse cytoplasmic immunostaining in some motor neurons. Patients that harbor mutations in *MATR3* exhibited the most intense nuclear immunoreactivity.

Objectives: Our goal was to characterize the function of wild-type and mutant Matrin 3 protein to help define its role in ALS.

Methods: Cell lines expressing each of the four mutations and wild type *MATR3* were created in HEK cells and Neuro2a neuroblastoma cells. These cell lines as well as tissue from ALS patients, controls and an ALS patient with a *MATR3* mutation were used in our studies. Immunoprecipitation was performed and proteins separated by gel electrophoresis. Gel slices were digested with trypsin and proteins identified by liquid chromatography tandem mass spectroscopy (LC-MS/MS).

Results: We identified 192 proteins that specifically interact with wild-type Matrin 3 using immunoprecipitation coupled to mass spectroscopy (IP-MS). Our IP-MS results confirmed a prior report suggesting Matrin 3 binds to TDP-43(2). We further demonstrated that the binding between Matrin 3 and TDP-43 is affected by some of the mutations in *MATR3*. A number of other proteins that function in RNA metabolism and translation also interact with Matrin 3. The Matrin 3 protein interactome is altered by mutations within *MATR3*. Cells that stably express wild type or mutant *MATR3* exhibit Matrin 3 immunostaining predominantly in the nucleus, similar to our findings in human tissue. We are also screening a panel of stressors to Matrin 3-expressing cells to determine changes in cell vulnerability caused by mutations in *MATR3*.

Discussion and conclusion: We recently identified mutations in the *MATR3* gene linked to familial ALS. Our current study creates a model system to investigate the function of Matrin 3 and how disease associated mutations alter protein function. We performed IP-MS to define the protein interactome of Matrin 3 and confirmed direct interactions of Matrin 3 to TDP-43. We have also explored how disease associated mutations in Matrin 3 alter protein interactions. Our studies will lead to a better understanding of how Matrin 3 functions in RNA metabolism and its role in the pathophysiology of ALS.

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P213 DEVELOPMENT OF AN ANTISENSE OLIGONUCLEOTIDE-BASED METHOD TO MANIPULATE RNA EDITING OF AMPAR SUBUNITS

CHAYTOW H, POPPLEWELL L, DICKSON G, CHEN P

Royal Holloway University, London, UK

Email address for correspondence: Helena.Chaytow.2012@live.rhul.ac.uk

Keywords: calcium signalling, neurodegeneration

Background: AMPA receptors (AMPARs) are a subset of glutamate receptors composed of one or more of four different subunits (GluA1–4). AMPARs are generally permeable to calcium, unless the GluA2 subunit is present. AMPAR subunits undergo RNA editing at specific sites in their sequence, a post-transcriptional modification caused by deamination of an adenosine subunit. This forms inosine, which is read by the cell's translation machinery as guanosine therefore changing the codon, critical for the GluA2 subunit's impermeability to calcium. Studies have shown that reduced editing is found in spinal cord motor neurons of MND patients, and this may lead to their cell death via increased calcium influx (1). Deamination is carried out by a family of enzymes called Adenosine Deaminases Acting on RNAs (ADARs). ADAR2 has multiple alternatively-spliced variants within mammalian cells: some have been shown to reduce the efficiency of ADAR2's deamination. One such alternatively-spliced transcript contains the addition of an exon containing an AluJ sequence. We can interfere with endogenous exon splicing using Antisense oligonucleotides (ASOs); short complementary nucleotide sequences which can be used to prevent AluJ exon inclusion.

Objectives: We designed ASOs targeting the splice sites surrounding the AluJ-containing exon in ADAR2 and examined their effects on AluJ inclusion and the RNA editing process in mammalian cell lines.

Methods: HeLa, NSC34 and SHSY5Y cells were grown in culture and treated with ASOs ($n = 3$ per treatment group). HeLa cells do not endogenously express AMPARs, and so a plasmid containing a section of the GluA2 subunit was transfected into cells in order to measure RNA editing. Editing efficiencies were measured using a RT-PCR based assay on RNA extracts.

Results: Endogenous ADAR2 within HeLa cells exhibited an inclusion rate of the AluJ exon of 59.23% (± 0.62) within ADAR2 transcripts (ie, there is endogenous exon skipping of around 40%). When exposed to 2 μM of ASO, exon skipping was enhanced to 99.37% (± 0.41, $p < 0.05$). Exclusion of the AluJ exon in ADAR2 significantly increases editing of a minigene containing a section of GluA2 from 23.1 ± 1.07% to 29.5 ± 0.98% ($p < 0.05$). We are now examining the effects of our ASOs in neuronal-like cell lines (NSC34s and SHSY5Ys) known to endogenously express the GluA2 subunit.

Discussion and conclusion: This shows promising preliminary data for the use of ASOs to alter RNA editing of AMPARs. Ultimately this ASO will be tested in primary neuronal cultures, where this increased editing efficiency can be enhanced and may prove to be beneficial to neuronal viability.

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P214 FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS-ASSOCIATED PROTEINS FORM AGGREGATES VIA DISTINCT PATHWAYS IN CELLS

YERBURY J[1,2], FARRAWELL N[1,2], LAMBERT-SMITH I[1,2]

[1]*University of Wollongong,* [2]*Illawarra Health & Medical Institute, Wollongong, NSW, Australia*

Email address for correspondence: jyerbury@uow.edu.au

Keywords: protein aggregation, protein homeostasis, cell biology

Background: ALS pathology is characterised by intraneuronal protein deposits including ubiquitylated round, conglomerate or skein-like inclusions. The composition of ubiquitylated inclusions varies considerably depending on whether the disease is sporadic or familial and the genetics of the familial forms. Both FUS and SOD1 mutations cause ALS with FUS (1, 2) and SOD1 positive inclusions (3), respectively. However, most other cases have inclusions positive for TDP-43 (3). Given that protein aggregation is linked to toxicity we sought to understand the types of aggregates formed by TDP-43, FUS and SOD1.

Objectives: In cells, protein aggregation is thought to procede via formation of juxtanuclear quality control compartments (JUNQ) or insoluble protein deposits (IPOD) (4, 5). This study aimed to characterize the aggregation pathways of mutant TDP-43, FUS and SOD1 in cells.

Methods: Cell models were created in NSC-34 cells using mutant TDP-43, FUS and SOD1 fused to GFP. Inclusion formation was followed by GFP foci, ubiquitin-RFP and with a Htt_{ex1}-46Q-RFP reporter of iPOD formation. Microtubules were destabilized by treatment with nocodazole. Inclusion mobility was measured using fluorescence recovery after photobleaching (FRAP) on a Leica SP5 scanning confocal microscope.

Results: SOD1 JUNQ-like inclusions are always positive for ubiquitin, do not co-localize with Htt, contain a mobile fraction and are dependant on microtubules as found previously (4, 5). FUS inclusions contain a mobile fraction, do not require microtubules and co-localize to Htt. In contrast TDP-43 inclusions are not dependant on microtubules, are immobile, but do not co-localize to Htt. Moreover, TDP-43 and FUS inclusions progressively co-localise with ubiquitin with time and are adjacent to LC3-positive foci.

Discussion and conclusion: These data suggest that the properties of TDP-43-, FUS- and SOD1-inclusions and the pathways through which they form are distinct. In addition, we propose that TDP-43 inclusions represent a newly identified mechanism of protein aggregation in the cell. This will have implications for potential therapeutics targeting protein aggregation pathways in ALS.

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P215 ALS-ASSOCIATED MUTANTS OF SOD1, TDP-43, FUS, C9ORF72 AND UBQLN2 DISRUPT PROTEOSTASIS IN NEURONAL CELL CULTURE

LAMBERT-SMITH I[1,2], SAUNDERS D[2], YERBURY J[1]

[1]*University of Wollongong, Wollongong, NSW, Australia,* [2]*Garvan Institute of Medical Research, Sydney, NSW, Australia*

Email address for correspondence: ials563@uowmail.edu.au

Keywords: protein homeostasis, ubiquitin, genetic mutations

Background: The ability of motor neurones to maintain protein homeostasis (proteostasis) is significantly compromised in ALS. Proteostasis maintenance is necessary for proper cellular function, and clear evidence for its disruption lies in the common occurrence of ubiquitin-positive inclusions within affected motor neurones in the various genetic forms of ALS.

Objectives: This study aimed to investigate the proteostasis disruptions caused by familial ALS (fALS)-associated gene mutants in a neuronal cell culture model, using reporter substrates for the ubiquitin-proteasome system (UPS) and cellular chaperone activity.

Methods: NSC-34 cells were transfected with mutants of SOD1 (A4V), TDP-43 (M337V), FUS (R495X), C9ORF72 (38x and 72x expansions of the GGGGCC repeat), and UBQLN2 (P525H) fused to GFP. Inclusion formation was followed by either GFP foci presence in cells or by co-transfection with ubiquitin-RFP. NSC-34 and SH-SY5Y cells were also co-transfected with the GFP fusion proteins and a tomato-CL1 reporter of UPS activity. The CL1 degron sequence targets it for proteasome degradation (1); any blockade of this system results in accumulation of the tomato fluorescent protein signal. Additionally, SOD1 and TDP-43 fused to tomato were co-transfected with mutants of firefly luciferase fused to GFP (FLuc-GFP), which act as reporters of the proteostasis environment (2); the FLuc mutations destabilise the protein, therefore any perturbations in the chaperone-mediated folding environment will result in an inability of chaperones to monitor and refold the FLuc reporters.

Results: In cells expressing the fALS-associated mutants we observed differences in the timing of ubiquitin localisation to inclusions, potentially reflecting different mechanisms underlying inclusion formation. High level expression of SOD1, C9ORF72 and UBQLN2 caused tomato-CL1 accumulation. This accumulation was particularly striking in cells expressing mutant TDP-43 and FUS. In addition we found SOD1 expression caused an increase in mutant FLuc aggregation, indicating a deficiency in the availability of chaperones and therefore a reduction in global proteostasis capacity.

Discussion and conclusion: These data provide insight into the ways in which these fALS-associated genetic mutations lead to disruptions in motor neurone ability to maintain proteostasis. In particular we have discovered that these mutations compromise the UPS. Protein quality control systems involve many different proteins with defined roles. It will be important to closely examine the specific perturbations caused by the fALS-mutants and assess whether these changes in proteostasis result in the same motor neurone pathology that characterises this disease.

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P216 STRUCTURAL AND DYNAMIC CHARACTERISATION OF TDP-43 USING NMR SPECTROSCOPY

WEISE A[1], KIRKPATRICK JP[1], ROBERTSON AL[1], NG JSW[2], KUMITA JR[2], SCOTTER EL[3], SHAW CE[3], DOBSON CM[2], CHRISTODOULOU J[1], CABRITA LD[1]

[1]*University College London, Institute of Structural and Molecular Biology and Birkbeck College, School of Crystallography, London, UK,* [2]*University of Cambridge, Department of Chemistry, Cambridge, UK,* [3]*King's College London, Institute of Psychiatry, London, UK*

Email address for correspondence: annika.weise.11@ucl.ac.uk

Keywords: TDP-43, structure, NMR

High-resolution structural information describing TDP-43 is scarce despite the clinical relevance and the powerful prospect of such data significantly aiding the understanding of this protein. Composed of an NLS-containing N-terminus and two RNA-binding domains (RRM1, RRM2) followed by a glycine-rich C-terminus ($CTD_{274–414}$), TDP-43 forms toxic aggregates as part of its disease-related pathology that lead to ALS and FTLD.

The $CTD_{274–414}$ harbours almost all of the ALS-associated mutations and is predicted to be an intrinsically disordered protein (IDP) domain. Disorder however does not preclude the existence of an overall architecture as has been shown to be particularly relevant in other disease relevant IDPs such as α-synuclein and tau. We have developed an expression and purification strategy using *E. coli* to produce isotopically-labelled and monomeric material and used NMR to provide a detailed structural and dynamic description of aggregation-prone $CTD_{274–414}$ both in isolation and as it exists within living cells. These data are discussed in the context of our biophysical observations (using EM and ThT-binding) of the amyloid-like aggregates formed by this aggregation-initiating region of TDP-43.

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P217 PROFILIN 1 ASSOCIATES WITH STRESS GRANULES AND ALS-LINKED MUTATIONS ALTER STRESS GRANULE DYNAMICS

FIGLEY M[1], BIERI G[1], KOLAITIS R-M[2], TAYLOR JP[2], GITLER A[1]

[1]*Stanford University, Stanford, California, USA,* [2]*St. Jude Children's Research Hospital, Memphis, Tennessee, USA*

Email address for correspondence: figley@stanford.edu

Keywords: profilin, stress granules, ataxin-2(ATXN2)

Mutations in the PFN1 gene encoding profilin 1 are a rare cause of familial amyotrophic lateral sclerosis (ALS) (1). Profilin 1 is a well-studied actin-binding protein but it is unknown how PFN1 mutations cause ALS. The budding yeast, *Saccharomyces cerevisiae,* has one profilin ortholog.

We expressed the ALS-linked profilin 1 mutant protein in yeast, demonstrating a loss of protein stability, assayed by Western blot, and failure to restore temperature-sensitive growth to profilin mutant cells when compared to yeast expressing wildtype human profilin 1. Overexpression of wildtype profilin 1 or the ALS-linked mutants in a wildtype yeast background was tolerated, indicating a lack of gain-of-function toxicity in this model. This model provides for simple and rapid functional screening of novel ALS-linked profilin 1 variants.

To gain insight into potential novel roles for profilin 1, we performed an unbiased, genome-wide synthetic lethal screen with yeast cells lacking profilin (pfy1Δ). Using Synthetic Genetic Array analysis, we queried ~4800 nonessential yeast genes, identifying 127 genes which demonstrate a synthetic sick or lethal interaction with pfy1Δ yeast. Unexpectedly, deletion of several stress granule and processing body genes, including pbp1, were found to be synthetic lethal with deletion of pfy1.

Mutations in ATXN2, the human ortholog of PBP1, are a known ALS genetic risk factor, and ataxin 2 is a stress granule component in mammalian cells (2, 3). Given this genetic interaction and recent evidence linking stress granule dynamics to ALS pathogenesis (4), we hypothesized that profilin 1 might also associate with stress granules. Here we report that profilin 1 and related protein profilin 2 are novel stress granule-associated proteins as assessed by immunocytochemistry in mouse primary cortical neurons (cultured from E18 embryos) and in human cell lines *in vitro* (HeLa and U2OS) in response to various stressors (heat shock, oxidative stress induced by arsenite, and endoplasmic reticulum stress induced by DTT).

We also report that ALS-linked mutations in profilin 1 alter its stress granule dynamics. When overexpressed by transient transfection in U2OS cells, several of the mutant proteins are defective at localizing to arsenite-induced stress granules (C71G, M114T, G118V mutants, $p = < 0.001$) or being cleared from heat shock-induced stress granules after stress removal (E117G, $p = < 0.01$) when compared to wildtype profilin 1 ($n = 100$ transfected cells/condition and 3 experiments). This study provides further evidence for the potential role of stress granules in ALS pathogenesis.

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P218 HSPB8 IS RECRUITED INTO STRESS GRANULES UPON STRESS AND PROTECTS AGAINST TDP-43 MEDIATED TOXICITY *IN VIVO* IN *DROSOPHILA MELANOGASTER*

SEGUIN SJ[1], MORELLI FF[1], DIACCI C[1], GANASSI M[1], POLETTI A[2], CARRA S[1]

[1]*University of Modena and Reggio Emilia, Modena, Italy,* [2]*University of Milan, Milan, Italy*

Email address for correspondence: serena.carra@unimore.it

Keywords: protein quality control, stress granules, HSPB8

Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease comprising clinically indistinguishable sporadic (s) and familial (f) forms, associated with a number of genes (SOD1, TDP-43, FUS, UBQLN2, VCP, FIG4, CHMP2B, SQSTM1, C9ORF72). Most ALS proteins are involved in protein degradation, ubiquitin proteasome pathway (UPP) and autophagolysosomal pathway (APLP), RNA processing or stress granule (SG) response. In ALS, motoneurons accumulate protein aggregates that contain RNA-binding proteins markers of SGs. Both aggregated proteins and persistent SGs are cleared by autophagy (1). Thus, proteostasis (mediated by the protein quality control, PQC) and ribostasis (involving SGs) may be interconnected and their imbalance may participate in ALS. Thus, upregulation of key players of the PQC, including molecular chaperones (heat shock proteins), which can assist the autophagy-mediated degradation of specific clients, thereby restoring proteostasis (and indirectly, ribostasis), may be beneficial in ALS.

Objectives: We previously published that overexpression of the small heat shock protein HSPB8 protects against TPD-43 mediated toxicity in immortalized motoneurons. In fact, HSPB8 facilitates the autophagy-mediated degradation of truncated TDP-43, thereby decreasing its cytosolic aggregation (2). Here we further investigated HSPB8 mode of action, focusing on its potential role both at SGs and protein aggregates. We also investigated the protective role of HSPB8 against TDP-43 mediated toxicity *in vivo* in *Drosophila melanogaster*.

Methods: For the *in vitro* work, HeLa cells were treated with SG inducing agents, including arsenite, MG132, heat shock. Cells were fixed and HSPB8 subcellular localization, as well as SG formation was investigated by immunofluorescence using specific antibodies. HSPB8 colocalization with mutated FUS containing SGs was studied in HeLa cells transfected with HA-tagged R518K FUS. For the *in vivo* work, the following transgenic flies were used: GMR-mut-NLS-TDP-43/CyO; UAS-HSP67Bc/TM3; UAS-HSPB8/CyO and UAS-HSPB8/TM3. Flies were grown and crossed at 25°C.

Results: We found that HSPB8 is recruited into SGs upon various stress. HSPB8 also colocalizes with mutated FUS at SGs, suggesting that it may act both at the level of protein aggregates (previous results) and SGs. Next, we tested HSPB8 protective role *in vivo*, using a Drosophila model expressing a NLS-TDP-43 mutant that accumulates in the cytosol, thereby causing eye degeneration (kindly provided by Prof. JP Taylor). We found that overexpression *in vivo* of both human HSPB8 and its Drosophila functional ortholog HSP67Bc protects against TDP-43 mediated toxicity. This is associated with a decrease in NLS-TDP-43 levels, supporting HSP67Bc/HSPB8 role in facilitating client degradation.

Discussion and conclusion: Our results support that HSPB8 may act both at the level of aggregate-prone proteins as well as SGs, facilitating the degradation of mutant ALS proteins (eg, TDP-43) and decreasing their cytosolic accumulation, thereby exerting protective functions.

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P219 TDP-43 REGULATES RNA GRANULE DYNAMICS AND INTERACTION: A NOVEL ELEMENT OF ALS PATHOGENESIS

AULAS A[1], KHALFALLAH Y[1], MOHAMED N-V[1], GKOGKAS C[2], CARON G[1], DESTROISMAISONS L[1], SONENBERG N[2], LECLERC N[1], PARKER JA[1], VANDE VELDE C[1]

[1]*Centre de recherche CHUM, Universite de Montreal, Montreal, Canada,* [2]*McGill University, Montreal, Canada*

Email address for correspondence: c.vande.velde@umontreal.ca

Keywords: TDP-43, RNA granules, translation

Background: Stress granules (SG) are an integral part of the cellular stress response and are a recent convergence point in studies related to the pathogenesis of Amyotrophic Lateral Sclerosis (ALS). RNA binding proteins, such as TDP-43, are now well linked to ALS, in both familial and sporadic contexts. We have previously reported TDP-43 to be an important regulator of the SG response. We hypothesize that motor neuron loss/dysfunction in ALS is due to a loss of normal TDP-43 function associated with SG dynamics.

Objective: To determine the role of TDP-43 in RNA granule dynamics.

Methods: Evaluate endogenous TDP-43 function via siRNA-mediated knockdown on RNA granules induced by oxidative stress as assessed by high resolution confocal microscopy and biochemical methods.

Results: TDP-43 depletion impairs secondary aggregation of SG such that SG size is significantly reduced. We have demonstrated this defect is via TDP-43 dependent expression of G3BP1. Here, we report that despite this defect, the recruitment of mRNA and several well-defined SG proteins is normal in TDP-43 depleted conditions. Moreover, the transient translational repression that characteristically accompanies SG formation also proceeds normally. However, TDP-43/G3BP1 depletion yields reduced basal translation despite elevated levels of mRNA and increased numbers of Processing Bodies (PB). The interaction of SG and PB during stress (docking) is thought to facilitate the bilateral exchange of mRNA and proteins. We find that docking is reduced by TDP-43 depletion and this correlates with poor mRNA protection. Both docking and mRNA protection can be rescued by exogenous expression of the TDP-43 target G3BP1.

Discussion and conclusion: We report a novel consequence of TDP-43 (via regulation of G3BP1) in the alteration of SG dynamics and define SG size as a determinant of SG-PB docking and mRNA preservation. This offers a new pathway for ALS pathogenesis.

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P220 EVIDENCE THAT B-METHYLAMINO-L-ALANINE (BMAA) CAN GENERATE 'PRIONOID' PROTEINS THAT CAUSE CHRONIC PROTEOTOXIC STRESS *IN VITRO*

RODGERS K[1], DUNLOP R[1], MAIN B[1], GUILLEMIN G[2]

[1]*University of Technology, Sydney, Australia,* [2]*Macquarie University, Sydney, Australia*

Email address for correspondence: kenneth.rodgers@uts.edu.au

Keywords: protein misfolding, environmental toxin, BMAA

Background: Gene-environment interactions are implicated in sporadic MND but the identities of the environmental triggers remain elusive. The high incidence of the ALS-Parkinsonism-Dementia complex reported on Guam has been linked to exposure to the cyanobacteria-derived amino acid β-methylamino-L-alanine (BMAA). Our recent observation that BMAA can be mistakenly incorporated into proteins in place of L-serine *in vitro* causing protein misfolding in cells (1) provides a mechanism whereby exposure to BMAA could increase the burden of protein aggregates in neurons over the lifetime of an individual; a process that might be further increased in individuals carrying mutations in aggregate-prone proteins.

Objectives: We test the hypothesis that incorporation of BMAA into protein can generate misfolded 'prionoid' proteins in cells capable of initiating further protein aggregation, establishing a chronic proteotoxic stress in cells, a decline in cell function and apoptosis.

Methods: Cells examined were MRC-5 (fibroblasts), SH-SY5Y (neuroblastoma cells) and human primary neurons (foetal brain). Proteomic analysis was by 2D-gel electrophoresis and ESI/MS/MS and/or MALDI/TOF. Protein quantification was by western blotting, mRNA by RT-qPCR and assessment of apoptosis and necrosis by caspase 3 and MTT/LDH activity, respectively.

Results: We compared the proteome of BMAA-treated cells to that of control cells. 10 proteins were identified that were significantly increased ($p < 0.001$) after BMAA exposure (300μM) including proteins involved in the ER stress response (Grp78 and calnexin) and the heat shock response (Hsc70 and HSP90). In support of these data, we demonstrated that the transcription of heat shock factor 1, a primary regulator of the heat shock response, was increased with BMAA but remained at basal levels on co-treatment with L-serine. Markers of ER stress were rapidly increased in response to BMAA by RT-qPCR (CHOP, ATF4). Changes were also evident in proteolytic activity after prolonged exposure to BMAA (500μM, 72 hours).

Discussion and conclusion: These studies show that exposure of a range of cells to BMAA produces changes consistent with the presence of misfolded proteins in the ER and activation of a global heat shock response in the cell. Since most neurodegenerative diseases are associated with protein misfolding, the presence of a non-protein amino acid, such as BMAA, in the polypeptide chain could increase the rate of misfolding and aggregation of vulnerable proteins, triggering ALS, Parkinsonism, and dementia in susceptible individuals, as was evident on Guam. The ability of the cell to handle misfolded proteins could decrease as the nervous system ages and the function of the defence systems and proteolytic machinery decline. This would provide a link between environmental factors, genetic susceptibility and ageing (gene-time-environment model).

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P221 INHIBITION OF DYNEIN ATPASE ACTIVITY REDUCES AGGREGATION OF MISFOLDED PROTEIN RESPONSIBLE FOR SBMA AND ALS

CRISTOFANI R[1], GIORGETTI E[1,2], CRIPPA V[1], BONCORAGLIO A[1,3], CICARDI ME[1], RUSMINI P[1], CARRA S[4], POLETTI A[1]

[1]*Dipartimento di Scienze Farmacologiche e Biomolecolari (DISFEB) e Centro di Eccellenza sulle Malattie Neurodegenerative (CEND), Università degli Studi di Milano, Milani, MI, Italy,* [2]*Department of Pathology, University of Michigan, Ann Arbor, Michigan, USA,* [3]*Department of Cell Biology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands,* [4]*Dipartimento di Scienze Biomediche, Metaboliche e Neuroscienze, Università degli Studi di Modena e Reggio Emilia, Modena, Italy*

Email address for correspondence: angelo.poletti@unimi.it

Keywords: retrograde transport, autophagy, proteasome

Background: Spinobulbar muscular atrophy (SBMA) and amyotrophic lateral sclerosis (ALS) are characterized by the presence of aggregates of aberrant proteins (androgen receptors (AR), superoxide dismutase 1 (SOD1), TAR DNA binding protein 43 (TDP-43), etc.) with abnormal conformations (misfolding). These accumulate into aggregates because the protein quality control system is not sufficiently active for their correct removal. Dynein motor complex seems to play a crucial role to maintain an efficient clearance of misfolded proteins by autophagy. Dynein mediated the transport of misfolded proteins/aggregates near microtubule organization center (MTOC). In addition dynein allows autophagosome nucleation by binding Vps34-Beclin1-Ambra1 complex, and assists the fusion of autophagosome to lysosome.

Objectives: In this study we aimed to identify the contribution of dynein motor complex in the removal of misfolded proteins which accumulate in motoneuron diseases.

Methods: We used NSC34 cells transfected with mutant SBMA-AR, G93A-SOD1 and ΔC-TDP-43. Dynein inhibition was obtained using the selective dynein ATPase inhibitor EHNA at 0,1mM for 48h. Protein accumulation was quantified by retained protein on cellulose acetate membrane in filter retardation assay (FRA). Autophagy activity was analyzed using the LC3 marker by western blotting. Misfolded proteins rerouting to proteasome was analyzed measuring the BAG1/BAG3 ratio in RT-qPCR.

Results: Inhibition of dynein, drastically reduce the LC3II/LC3I ratio when autophagy is induced by trehalose. Immunofluorescence on NSC34 transfected with SBMA-AR showed that dynein is sequestered into mutant SBMA-AR aggregates. Unexpectedly, EHNA reduces aggregates of mutant proteins in FTA also in presence of autophagy inhibitor (3-MA), but not in presence of proteasome inhibitor (MG132). In order to confirm these data we produced an inducible stable transfected GFP-SBMA-AR cell line. Also in this case EHNA treatment reduces aggregate retained in FRA. Moreover, EHNA treatment increases the levels of mutated AR in PBS and Triton-X100 fraction in progressive extraction. When we analyzed the mRNA levels of the co-chaperones BAG1 and BAG3, which route misfolding proteins to proteasome degradation or chaperone-mediated-autophagy (BAG1) and autophagy (BAG3), we found that BAG1/BAG3 ratio is increased after EHNA treatment.

Conclusion: These data suggest that dynein impairment, which results in autophagy blockage, also reduces aggregation of misfolding proteins involved in MNDs, by increasing their solubility and possibly through induction of alternative degradative pathways.

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P222 COORDINATED ACTIVATION OF AUTOPHAGY AND CATHEPSIN-MEDIATED PROTEOLYSIS INHIBITION IS ESSENTIAL FOR NEUROPROTECTION BY CYSTATIN C AGAINST MUTANT SOD1-MEDIATED TOXICITY

WATANABE S[1], WAKASUGI K[2], YAMANAKA K[1]

[1]*Department of Neuroscience and Pathobiology, Research Institute of Environmental Medicine, Nagoya University, Nagoya, Aichi, Japan,* [2]*Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Meguro, Tokyo, Japan*

Email address for correspondence: swtnb@riem.nagoya-u.ac.jp

Keywords: cystatin c (CysC), cathepsin b, AMP-activated protein kinase (AMPK)

Background: Recent studies have revealed that Cystatin C (CysC), an endogenous cysteine protease inhibitor and a major component of Bunina bodies in sporadic ALS, plays neuroprotective roles in various neurodegenerative diseases. We previously presented CysC reduces mutant SOD1-mediated toxicity through induction of autophagy, however, the precise neuroprotective mechanism of CysC was yet unidentified.

Objectives: The aim of this study is to reveal the mechanisms of neuroprotective activity of CysC.

Methods: We treated the neuro2a cells expressing wild-type or mutant SOD1 with several pharmacological agents or recombinant proteins as listed below: AICA-ribose (AICAR), an AMP-activated kinase (AMPK) agonist and an autophagy inducer; CA-074 methyl ester, a membrane-permeable cathepsin B (CatB) specific inhibitor; W106G CysC, CysC mutant lacking the CatB inhibitory activity. The cell viability was measured by MTS assay.

Results: Administration of CysC into neuro2a cells induced phosphorylation of AMPK and inhibited CatB aberrantly activated by mutant SOD1. Unexpectedly, AICAR treatment or W106G CysC did not rescue the cells regardless of autophagy induction. CA-074 also failed to protect the cells. Therefore, both autophagy induction and CatB inhibition were essential for the neuroprotection by CysC. In addition to this, CysC was leaked from lysosomes and formed intracellular aggregates dependent on oxidative stress, implying a relationship between the neuroprotective activity of CysC and Bunina body formation.

Discussion and conclusion: Our findings indicate that the neuroprotective property of CysC was dependent on the coordination of two distinct pathways: autophagy induction through AMPK activation and inhibition of CatB.

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P223 THE INTERPLAY BETWEEN ENDOPLASMIC RETICULUM STRESS AND NF-KB IN ALS

PRELL T, JINGYU L, TADIC V, LAUTENSCHLÄGER J, GUNKEL A, HAMMER N, WITTE O, GROSSKREUTZ J

Hans Berger Department of Neurology, Jena, Germany, Germany

Email address for correspondence: tino.prell@med.uni-jena.de

Keywords: ER stress, neuroinflammation, SOD1^{G93A}

Background: Recent studies have indicated that endoplasmic reticulum (ER) stress is involved in the pathogenesis of ALS (1, 3). ER stress occurs when misfolded proteins accumulate in the ER (2). New observations-particularly those stemming from cancer cell lines-suggest that ER stress and the subsequent engagement of the unfolded protein response (UPR) can activate the NF-κB pathway.

Objectives: We attempted to determine whether ER stress can activate the NF-κB pathway in murine motor neurons and vice versa.

Methods: We used an established motor neuron culture system derived from embryonic mouse ventral spinal cords and the mouse motor neuronal cell line NSC-34, both of which overexpress the G93A mutant of human SOD1 (SOD1^{G93A}). Cells were treated with an ER stressor (tunicamycin) and an inflammatory stimulus (lipopolysaccharide, LPS), and the subsequent activation of the UPR and the NF-κB pathway was measured by immunhistochemistry and western blotting (NF-κB, X-box binding protein 1, basic leucine-zipper transcription factor 6, and phosphorylated eukaryotic initiation factor-2α).

Results: We observed a constitutive expression of NF-κB in motor neurons and motor-neuron-like cells and the expression of NF-κB was enhanced in the presence of SOD1^{G93A}. The stimulation of NSC34 cells with LPS did not activate the UPR. Moreover, the stimulation of the glia-motor neuron culture with LPS did not activate the UPR. The chemical induction of the UPR by tunicamycin was accompanied by the activation of NF-κB in motor neurons and NSC34 cells.

Conclusion: Our data link two important pathogenic mechanisms of ALS, ER stress and NF-κB signalling, in motor neurons. The increased NF-κB activity that was observed here in the affected SOD1^{G93A} neurons may serve a neuroprotective function. Similar to the UPR, it can be an early protective response to ongoing disturbances in the ER-mitochondria calcium cycle in ALS.

Acknowledgment: This research is supported by a BMBF (the Bundesministerium fürBildung und Forschung) grant PYRAMID in the framework of the ERANET E-RARE program (http://www.e-rare.eu) and was undertaken in cooperation with the BMBF funded MND-NET.

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P224 FIBROBLASTS FROM SALS PATIENTS DO NOT SHOW AGE RELATED CHANGES IN BIOENERGETIC PROPERTIES AND MITOCHONDRIAL MORPHOLOGY

ALLEN S, DUFFY L, SHAW PJ, GRIERSON A

University of Sheffield, Sheffield, UK

Email address for correspondence: s.p.allen@sheffield.ac.uk

Keywords: mitochondria, metabolism, aging

Background: Metabolic dysfunction plays a key role in amyotrophic lateral sclerosis (ALS) disease progression and has been observed in several cellular and animal models of the disease (1, 2). Evidence suggests that mitochondria play an important part in aging, which is a crucial risk factor in neurodegenerative disorders (3). Peripheral tissues such as fibroblasts have been observed to recapitulate pathophysiological abnormalities observed in the CNS (4, 5).

Objectives: To ascertain whether fibroblasts isolated from sporadic ALS patients show the defects in energy metabolism reported in the CNS and to ascertain how these patients respond to aging at a metabolic levels compared to controls.

Methods: Using mitochondrial morphology analysis, ATP assays and a XF24 bioanalyser, which simultaneously measures the two major energy producing pathways of the cell, mitochondrial respiration and glycolysis in real-time. We assessed the effect of metabolic dysfunction in SALS patient fibroblasts compared to controls and how ALS affects the metabolic response to aging.

Results: SALS fibroblasts show age independent reduced mitochondrial coupled respiration compared to controls ($p = 0.043$). Unlike SALS cases, control fibroblasts show increased mitochondrial spare respiratory capacity (SRC) with age ($p = 0.025$), decreased glycolytic flux ($p = 0.025$) and an increase in the OCR/ECAR ratio ($p = 0.001$) indicating an increased reliance on oxidative phosphorylation to meet energy needs. Unlike controls SALS fibroblasts show increased proton leak with age ($p = 0.053$) indicating damaged mitochondria. The oxphos: glycolysis ATP ratio tended to decrease with age in SALS cases but not controls. Mitochondrial network complexity increased with age in controls ($p = 0.03$) but not in SALS cases. The older SALS cases (70 years and above) had significantly reduced SRC and oxphos: glycolysis ATP ratio and also displayed increased glycolysis compared to controls.

Discussion and conclusion: Mitochondrial morphological and bioenergetic analysis, indicate that with age, control fibroblasts can rely more on mitochondrial function to meet energy demands. SALS fibroblasts are unable to do this as uncoupling and mitochondrial fragmentation increases with age in sporadic ALS. Older SALS cases display the greatest mitochondrial bioenergetic abnormalities and shift to a more glycolytic energy state. This highlights that from a mitochondrial functional perspective, older ALS patients are unable to cope with the aging process as well as controls and from a glycolytic metabolic perspective, SALS cells have an altered metabolic aging profile.

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P225 PGC-1ALPHA EXPRESSION AND STIMULATION IN ALS RELEVANT TISSUES AND CELLS

BAYER H, BARTECZKO L, HEINRICH M, MERDIAN I, WEYDT P, WITTING A

Experimental Neurology, Ulm, Germany

Email address for correspondence: hanna.bayer@uni-ulm.de

Keywords: PGC-1alpha, lactate-shuttle, metabolism

Background: The transcriptional co-activator PGC-1alpha is an important regulator of mitochondrial activity and biogenesis in many metabolically active tissues, including brain. Clinical and experimental evidence suggests that impaired function or activity of PGC-1α contributes to the pathogenesis of neurodegenerative disease spectrum disorders, including ALS. Of note, genetic variants of a brain-specific promoter modify age of onset in human ALS. We found that the brain-specific PGC-1α isoforms are differentially expressed in neurons vs. glial cells (1), suggesting that neurons and glia cells are metabolically differently regulated. According to the Lactate-Shuttle hypothesis lactate is released by glia cells to nourish neurons, whereas glutamate is released by neurons to stimulate lactate production by glia cells.

Objectives: Here we first examine the expression of brain-specific PGC-1α isoforms during disease progression in different brain regions and spinal cord of the ALS mouse model, B6SJL-Tg (SOD1^{G93A})1Gur/J. Second, we analyzed the activation of PGC-1α in primary murine cell cultures of neurons and glia cells, including oligodendrocytes, astrocytes and microglia.

Methods: Tissues from SOD1 mice were harvested at 60 days (preclinical), at 100 days (onset) and at 130 days (end-stage). Primary murine cells were stimulated with lactate or glutamate. The expression of the different PGC-1α isoforms and

enzymes involved in glycolysis and Krebs cycle was quantified by Real-time PCR.

Results: We showed that PGC-1α is significantly reduced in ALS end-stage mice in different brain regions and the spinal cord. PGC-1α is also differentially expressed in the different cell types, eg, neurons and oligodendrocytes, express much higher levels of the novel brain-specific isoforms of PGC-1α. Treatment of primary neurons with lactate leads to an increase in PGC-1α whereas treatment of oligodendrocytes with glutamate results in a decrease of PGC-1α and pyruvate dehydrogenase.

Discussion and conclusion: The different expression levels might reflect the specific metabolic needs of the different cell types and their different role in the contribution to the lactate shuttle. A reduced PGC-1α expression in ALS mice might therefore reflect an insufficient lactate shuttle, which has been shown to be of major interest to ALS.

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P226 MIRO1 EXPRESSION RESCUES ALS MUTANT SOD1-INDUCED INHIBITION OF ANTEROGRADE TRANSPORT OF MITOCHONDRIA

MOLLER A, DE VOS KJ

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

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

Keywords: mitochondria, axonal transport, SOD1

Background: Damage to axonal transport is an early event in ALS that may contribute to motor neuron degeneration. We have shown previously that familial ALS mutations in SOD1 and VAPB selectively reduce anterograde transport of mitochondria (1, 2).

Anterograde transport of mitochondria is mediated by the molecular motor kinesin-1 that attaches to mitochondria via the adaptor protein TRAK and the atypical Rho GTPase Miro1 (3, 4). Miro1 plays a central role in the regulation of mitochondrial axonal transport in response to Ca^{2+} and mitochondrial damage. Firstly binding of Ca^{2+} to the Miro1 EF-hand motifs halts anterograde transport of mitochondria by regulating the interaction of kinesin-1 with Miro1 such that either kinesin-1 binding to microtubules or to Miro1 itself is disrupted (5, 6). Secondly, PINK1 phosphorylates Miro1 in response to mitochondrial damage. This activates proteasomal degradation of Miro1 in a Parkin-dependent manner and as a result detaches kinesin-1 from mitochondria and arrests anterograde mitochondrial movement (7).

Objectives: Here we investigated the mechanisms underlying defective anterograde transport of mitochondria in mutant SOD1-related ALS.

Methods: Rat cortical neurons were transfected with EGFP, EGFP-tagged wild-type SOD1 or $SOD1^{G93A}$ and either empty vector, wild type Miro1 or the Ca^{2+} insensitive $Miro1^{E208K/E328K}$ mutant. Axonal transport of mitochondria labeled with DsRed-Mito was quantified from time-lapse recordings. The effect of ALS mutant SOD1 on Miro1 protein levels was investigated in HEK293 cells co-transfected with myc-Miro1 and either wild type or $SOD1^{G93A}$.

Results: $SOD1^{G93A}$ significantly decreased the total number of motile mitochondria by specific reduction of their anterograde transport; retrograde transport of mitochondria was not affected. This specific anterograde transport defect unbalanced axonal transport of mitochondria such that there was a significant shift toward net retrograde transport. Expression of either wild type Miro1 or $Miro1^{E208K/E328K}$ rescued this defect and restored the transport balance, suggesting $SOD1^{G93A}$ may affect Miro1 levels. In agreement, $SOD1^{G93A}$ significantly decreased Miro1 levels compared to wild type SOD1 in HEK293 cells.

Discussion and conclusion: These data indicate that Miro1 degradation may play a role in ALS mutant SOD1-induced defects in axonal mitochondrial transport.

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P227 PERTURBATION OF CITRIC ACID CYCLE AND EXCITATORY NEUROTRANSMISSION IN AN *IN VITRO* MODEL OF AMYOTROPHIC LATERAL SCLEROSIS EXPOSED TO OXIDATIVE STRESS

VEYRAT-DUREBEX C[1,2], CORCIA P[1,3], PIVER E[4], EMOND P[1,5], DANGOUMAU A[1], VOURC'H P[1,2], DEVOS D[6], ANDRES CR[1,2], BLASCO H[1,2]

[1]*UMR INSERM U930, Tours, France,* [2]*Laboratoire de Biochimie et Biologie moléculaire, CHRU de Tours, Tours, France,* [3]*Service de Neurologie, CHRU de Tours, Tours, France,* [4]*INSERM U966, Tours, France,* [5]*PPF-ASB, Université François Rabelais, Tours, France,* [6]*EA1046, Faculté de médecine de Lille, Lille, France*

Email address for correspondence: charlotte.veyrat-durebex@univ-tours.fr

Keywords: co-culture, oxidative stress, metabolism

Background: Although genetic and environmental factors have been associated with amyotrophic lateral sclerosis (ALS), pathophysiology of ALS remains unknown in the majority of cases. Preliminary results from our laboratory on CSF components of ALS patients indicate a potential modification of energetic substrates consumed by the central nervous system (1).

Objectives: In order to improve the knowledge of the pathophysiology of this disease and associated metabolic alterations, we developed a metabolomic approach on a cellular model

reproducing genetic (mutation in SOD1 gene) and environmental (oxidative stress) conditions of ALS.

Methods: A co-culture model of mouse astrocytes (C8-D1A cell line) and motor neurons (NSC-34 cell line) expressing or not human SOD1 protein wild-type or G93C mutant were exposed to oxidative stress (menadione 10 μM). The two cell types were collected at 3 times post-stress (for 48h). Analyses of intracellular media were performed by gas chromatography coupled to mass spectrometry after extraction (methanol/water/chloroform) and derivation (oxymation and silylation) of intracellular metabolites. Data were analyzed using multivariate analysis (OPLS-DA) using Simca P+ software (Umetrics, Umea, Sweden).

Results: Twenty six metabolites observed in GC-MS were used in statistical models. Preliminary analysis of the results showed a difference of metabolism between the two cell types leading to a separate statistical analysis of the co-cultured astrocytes and motor neurons. We also noticed an influence of genetic and time on metabolism. We observed a different metabolism in the 48h of stress between stressed and unstressed cells, with different discriminant metabolites depending on cell type and genetic conditions. Thus we showed that stressed cells no over-expressing SOD1 have significant higher concentrations of glutamate and aspartate (astrocytes) and threonine, serine and aspartate (motor neurons). Meanwhile, stressed cells over-expressing mutant SOD1 have significant higher concentrations of glycine (astrocytes) and fumarate and valine (motor neurons).

Discussion and conclusion: Preliminary results from this study revealed the feasibility of a metabolomic approach on a cellular model of ALS. We brought out the potential disruption of citric acid cycle and excitatory transmission under oxidative stress in ALS, which joined the assumptions already raised in this disease. These data could open perspective of functional, genomic and transcriptomic approaches focused on metabolites identified in this study and help to better understand mechanisms of the disease.

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P228 SYNAPTIC MECHANISMS UNDERLYING THE EXCESSIVE AND PRECOCIOUS GLUTAMATE RELEASE IN THE SPINAL CORD OF SOD1^{G93A} EXPERIMENTAL MICE

BONIFACINO T[1], MILANESE M[1], MUSAZZI L[2], TRECCANI G[2], GIRIBALDI F[1], USAI C[4], ONOFRI F[3], POPOLI M[2], BONANNO G[1,4]

[1]*Department of Pharmacy, Pharmacology and Toxicology Unit, University of Genoa, Genoa, Italy,* [2]*Department of Pharmacological and Biomolecular Sciences, Laboratory of Neuropsychopharmacology and Functional Neurogenomics, University of Milan, Milan, Italy,* [3]*Department of Experimental Medicine, Section of Physiology, University of Genoa, Genoa, Italy,* [4]*Institute of Biophysics, National Research Council, Genoa, Italy,* [5]*Center of Excellence for Biomedical Research, Genoa, Italy*

Email address for correspondence: bonanno@difar.unige.it

Keywords: glutamate release, excitotoxicity, synaptic proteins, SOD1^{G93A}

Background: Glutamate (Glu)-mediated excitotoxicity plays a major role in the degeneration of motor neurons (MNs) in amyotrophic lateral sclerosis (ALS) and reduced astrocytic uptake was suggested as a cause for the increased synaptic availability of Glu (1). On the basis of our studies, we have proposed that abnormal release may represent another source for excessive extracellular Glu levels (2, 3, 4). Acting at the altered Glu release mechanisms may represent a possible strategy for new therapeutic approaches to ALS (5).

Objectves: To investigate the molecular mechanisms supporting the excessive Glu exocytosis in experimental ALS.

Methods: 4 weeks or 17 weeks old mice were used. Nerve terminals (synaptosomes) were obtained from the spinal cord of control (SOD1) and pathologic (SOD1^{G93A}) mice. As a functional readout we measured glutamate release, by exploiting the superfusion technique. We also measured the intrasynaptic calcium concentration and the expression/activation state of synaptic proteins by confocal microscopy and western blot experiments.

Results: The spontaneous and the stimulus-evoked exocytotic Glu release are increased in SOD1^{G93A} mice, respect to controls, either at 4 weeks or 17 weeks of life. The expression of several synaptic proteins involved in neurotransmitter release does not show differences except for synaptotagmin which results over-expressed at the active zone in both 4 weeks and 17 weeks old ALS mice. Accumulation of high molecular weight SNARE complexes and disregulation of cytoskeletal proteins are also present in isolated nerve terminals from the spinal cord of SOD1^{G93A} mice. Increased pre-synaptic Ca^{2+} levels, over-activation of calcium/calmodulin-dependent kinase-II and ERK/MAP kinases, correlates with a hyperphosphorylation of synapsin-I at both early and late clinical stages of the disease. In line with these findings, release experiments highlight the involvement of the readily releasable pool of vesicles for the excessive Glu exocytosis.

Discussion and conclusion: Our results indicate a dysregulation of glutamate exocytosis in the spinal cord of symptomatic SOD1^{G93A} mice. This event is accompanied by marked changes in specific pre-synaptic molecular mechanisms that lead to a significant augmentation of the readily releasable pool of vesicles and determine a higher probability of these vesicles to fuse. The same synaptic alterations are present also in 4 weeks old ALS mice and represent a key feature in the early phase of the disease, thus playing a pivotal role in the aetiopathogenesis of the disease.

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P229 ASTROCYTE-DERIVED TGF-B1 ACCELERATES DISEASE PROGRESSION IN ALS MICE BY INTERFERING WITH NEUROPROTECTIVE FUNCTIONS OF MICROGLIA AND T CELLS

ENDO F[1], KOMINE O[1], WYSS-CORAY T[2], YAMANAKA K[1]

[1]*Nagoya University, Research Institute of Environmental Medicine, Nagoya, Japan,* [2]*Stanford University, Palo Alto, CA, USA*

Email address for correspondence: kojiyama@riem.nagoya-u.ac.jp

Keywords: astrocyte, TGF-β1, SOD1

Background: Elevated level of transforming growth factor-β1 (TGF-β1), an anti-inflammatory cytokine, has been observed in the peripheral blood and the cerebrospinal fluid of ALS patients. However, the exact role of TGF-β1 in ALS pathomechanism has not been elucidated.

Objective: The aim of this study is to uncover the role of TGF-β1, anti-inflammatory cytokine in the pathomechanism of ALS.

Methods: Expression of TGF-β1 and its downstream signaling were examined in multiple lines of mutant SOD1 mice. $SOD1^{G93A}$ mice were crossbred with GFAP-TGF-β1 mice, which moderately overexpressed TGF-β1 in astrocytes. The disease phenotype, the phenotypes of glia/immune cells, and the expression profiles of cytokines and neurotrophic factors were analyzed in $SOD1^{G93A}$/GFAP-TGF-β1 and $SOD1^{G93A}$ mice. Finally, the role of astrocytic mutant SOD1 in the level of TGF-β1 was analyzed in $LoxSOD1^{G37R}$ mice.

Results: We identify astrocytic TGF-β1 as determinant of disease progression through regulating the neuroprotective glia / immune response in ALS mice. We show that TGF-β1 level is elevated in astrocytes of symptomatic mutant SOD1 mice, and that astrocyte-specific overproduction of TGF-β1 in $SOD1^{G93A}$ mice accelerates disease progression in a non-cell autonomous manner with reduced IGF-I production in deactivated microglia and fewer infiltrated T cells with a dysregulated IFN-γ / IL-4 balance. In addition, the level of TGF-β1 was decreased when mutant SOD1 is deleted specifically from astrocytes in $LoxSOD1^{G37R}$ mice that are known to extend their life span. Moreover, expression levels of endogenous TGF-β1 in $SOD1^{G93A}$ mice negatively correlate with survival time. On the other hand, we also show that canonical TGF-β signaling within motor neurons of $SOD1^{G93A}$ mice is dysregulated regardless of exogenous TGF-β1.

Discussion and conclusion: These findings indicate that astrocyte-derived TGF-β1 accelerates disease through negatively regulating the neuroprotective inflammatory response by microglia and T cells. Furthermore, cell-type-specific dysregulation of TGF-β signaling is critical to the pathomechanism of ALS.

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THEME 10 *IN VIVO* EXPERIMENTAL MODELS

P230 COMPARISON OF ZEBRAFISH MODELS OF ALS

LISSOUBA A, ARMSTRONG G, DRAPEAU P

Research Centre of the University of Montréal Health Centre, Montreal, Quebec, Canada

Email address for correspondence: alexandra.lissouba@umontreal.ca

Keywords: TARDBP, RNA-sequencing, zebrafish

Background: ALS-causing mutations have been discovered in several genes involved in RNA metabolism, such as *TARDBP* (coding for TDP-43) and *FUS*, two structurally and functionally related DNA/RNA binding proteins. However, the pathological dysfunction caused by ALS mutations in these genes is unknown. Zebrafish are optimal for the development of genetic models of ALS; the zebrafish embryos develop externally and are optically transparent, allowing for fluorescent labelling of live cells. Additionally, their swimming behaviour is well characterized, electrophysiological recording in whole live embryos is easily obtained and genetic manipulations are highly feasible.

Objectives: Using next-generation RNA-sequencing to study the transcriptome modifications induced by ALS-causative mutations of FUS and TARBDP, using our different genetic models of ALS.

Methods: We have used zebrafish to generate genetic models of ALS. Overexpression models were generated by injecting human ALS-related mutant *TARDBP* mRNA or FUS mRNA into 1-cell stage embryos. A stable *TARDBP* transgenic line was also generated, with the human gene placed under the control of the heat shock inducible *hsp70l* promoter. We extracted high quality mRNA from larvae obtained for each of these models of ALS and performed RNA sequencing using the Illumina Hi-seq 2000 technology.

Results: In all these models, the embryos exhibited an abnormal touch-evoked escape response, with a reduction in the distance swam, the maximum velocity of swim and in the swim duration. The motoneurons of these embryos also had abnormal, overbranched axons. Over 40,000 transcripts were sequenced and some 26,000 had human homologs. Analyses using an adjusted p-value < 0.1 revealed that any single condition altered the level of expression (up or downregulated) of only 32 to 278 genes; 22 genes were upregulated and 14 genes downregulated in at least two of these conditions. As TDP-43 and FUS are implicated in RNA processing, we are currently performing a transcriptome-wide analysis of splicing.

Discussion and conclusion: Our preliminary results indicate that surprisingly few genes are commonly altered between these different models, helping to define potential disease-related targets. We are determining if these commonly modified genes or pathways could be common denominators of the ALS pathology by testing if their overexpression or downregulation in wild-type zebrafish embryos can cause an ALS phenotype similar to the one we observe in our genetic models of ALS. Additionally, we are testing by qRT-PCR if the hits found in our *TARDBP* and *FUS* models are also misregulated in the presence of other ALS-causing genes, such as *SOD1* and *C9ORF72*.

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P231 INTERACTIONS OF ENVIRONMENTAL NEUROTOXINS WITH SOD1 IN AMYOTROPHIC LATERAL SCLEROSIS IN A ZEBRAFISH MODEL

SHER R, POWERS S, LAVIN T, KWOK S, LOVEJOY E

University of Maine, Orono, ME, USA

Email address for correspondence: roger.sher@umit.maine.edu

Keywords: BMAA, zebrafish, SOD1^{G93A}

Background: Both genetic and environmental causes of amyotrophic lateral sclerosis (ALS) have been identified, and the scientific consensus is that a combination of gene-environment interactions are key for the development of ALS, but how either toxicants or genes lead to a disease mechanism is currently unknown. This represents a major gap in our understanding of the pathogenesis of ALS (1). A suite of environmental neurotoxins has been associated with the development of ALS, with epidemiological, clinical, and experimental evidence indicating that early developmental exposures to neurotoxins can have consequences for neurotoxicity later in life (2). Potentially, impairment of genes crucial to early neuronal differentiation makes neurons more susceptible to additional environmental disruptions, leading to late-onset disease (3). By determining cellular pathways involved in modifying neurological defects, both by toxicants and genetic influences, we hope to gain a better understanding of the root causes of this disorder.

Objectives: The zebrafish, *Danio rerio*, has been shown to be a robust model organism for modelling human neurodegenerative diseases, including ALS. Our research aims to study the intersection of environmental neurotoxins on motor neuron defects in a zebrafish model of ALS.

Methods: We have determined the impact of exposure to varying doses (0–25μg/L) of BMAA (β-methylamino-L-alanine) on early neurological defects in mutant SOD1-ALS zebrafish.

Results: We have found that: (i) Motor neuron length (30hpf) is decreased in control and SOD1^{G93A} fish when exposed to BMAA, but not in SOD1-wt overexpressing fish; (ii) SOD1^{G93A} embryos are more sensitive to lower doses of BMAA than are control fish, and (iii) 72hpf neuromuscular

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junction architecture is significantly altered in BMAA-exposed SOD1^{G93A} but not in SOD1-wt overexpressing embryos.

Discussion and conclusion: Our results indicate that genetic and environmental insults combine to facilitate neurological dysfunction in ALS, and that overexpression of wt-SOD1 may have protective effects against neurotoxin damage.

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P232 NEW INSIGHTS ON HEMATOPOIETIC STEM CELLS DIFFERENTIATION IN TRANSGENIC SOD1^{G93A} MICE

GASCO S[1], CALVO AC[1], RANDO A[1], OLIVÁN S[1], TOIVONEN JM[1], ESTEBAN PÉREZ J[2], ZARAGOZA P[1], GARCIA REDONDO A[2], OSTA R[1]

[1]*Laboratorio de Genética Bioquímica (LAGENBIO-I3A), Aragon's Institute of Health Sciences (IACS), Facultad de Veterinaria, Universidad de Zaragoza, Zaragoza, Spain,* [2]*Unidad de ELA, Instituto de Investigación Hospital 12 de Octubre de Madrid, SERMAS, Madrid, Spain, and Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER U-723), Spain. Edificio C, Madrid, Spain*

Email address for correspondence: accalvo@unizar.es

Keywords: hematopoietic stem cell, flow cytometry, SOD1^{G93A}

Background: Hematopoietic stem cells (HSC) are multipotent cells with self-renewal capacity that can give rise to their closest oligopotent hematopoietic progenitor cells, best known as Common Lymphoid Progenitors (CLP) and Common Myeloid Progenitors (CMP) (1). In this study we propose to investigate the frequencies of HSC, CLP and CMP in a murine model along disease progression.

Objectives: The main aim of this study was to characterize for the first time the frequency of HSC, CLP and CMP in serial blood extractions from transgenic SOD1^{G93A} mice.

Methods: Blood samples from transgenic mice were collected and treated to study the frequency of HSC, CLP and CMP by real time PCR and flow cytometry. The blood samples were first collected at the age of 30 days and then serial samples were obtained from each animal along disease progression till the end-point. Two-tailed t-Student tests were used to assess statistical significance between groups. A total of 40 mice were included in this study, 20 control and 20 transgenic mice of both sexes.

Results: The results obtained in serial blood samples suggested at transcriptional level, HSC were almost significantly activated in transgenic mice at terminal stage ($p = 0.06$). Interestingly in the flow cytometry study, the frequency of HSC was significantly increased since symptomatic stage to the end-point of transgenic mice ($p < 0.01$). However, the frequencies of CLP and CMP decreased along disease progression, starting at late ($p < 0.05$) and early symptomatic stage ($p < 0.05$), respectively.

Discussion and conclusion: The time dependent increase in HSC frequencies together with the time dependent decrease in CLP and CMP frequencies could suggest a time-point dependent differentiation of HSC to CLP and CMP, which could be influenced by the degenerative progression of the disease. This result is in accordance with recent studies that reveal a potential therapeutic effect based on stem/progenitor cell transplantation at presymptomatic or early symptomatic stages in animal models for the disease (2) and in ALS patients (3). The time dependent activation of HSC could shed light on the role of hematopoietic system in the progression of ALS and future therapeutic targets could be defined.

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P233 ANDROGEN RECEPTOR DYSREGULATION IN ALS MOUSE MODELS

SHEEAN RK, PERERA ND, WESTON R, TURNER BJ

Florey Institute of Neuroscience and Mental Health, Melbourne, Australia

Email address for correspondence: bradley.turner@florey.edu.au

Keywords: SBMA, androgen receptor, SOD1^{G93A}

Background: Spinal bulbar muscular atrophy (SBMA) is an adult-onset lower motor neuron disease affecting males that results from pathogenic androgen receptor (AR) expansion and inactivation. AR inactivation is linked to neurodegeneration by loss of AR trophic signalling, leading to transcriptional dysregulation of growth factors required for motor neuron survival, and nuclear accumulation of toxic AR-polyglutamine inclusions. Interestingly, AR is abundant on spinal and cranial motor neurons also affected in ALS. The increased incidence of ALS in males also implicates AR signalling in pathogenesis. We therefore sought to determine a potential role for AR dysregulation in ALS.

Objectives: 1) To investigate AR expression and distribution in spinal cords of transgenic SOD1^{G93A} and TDP-43^{A315T} mice with disease progression; 2) To determine the impact of androgen ablation on AR expression, localisation and motor neuron loss, in SOD1^{G93A} mice.

Methods: AR expression level was analysed in spinal cords of SOD1^{G93A} and TDP-43^{A315T} mice at presymptomatic and symptomatic ages compared to age-matched wild-type controls by Western blotting. AR localisation in NeuN, GFAP and CD11b-positive cells in spinal cord was established by immunohistochemistry. SOD1^{G93A} mice were sham operated or surgically castrated and studied for AR expression, localisation and motor neuron loss in spinal cords.

Results: AR protein was significantly and progressively downregulated by 50% in presymptomatic and 80% in symptomatic SOD1^{G93A} mouse spinal cords ($p < 0.05$). AR was abnormally depleted from cell bodies and accumulated in proximal axons of motor neurons in SOD1^{G93A} mice, but not in astrocytes or microglia. Androgen ablation in SOD1^{G93A} mice significantly enhanced AR depletion in motor neurons ($p < 0.01$). AR was also depleted in spinal motor neurons of presymptomatic TDP-43^{A315T} mice.

Discussion and conclusion: We show for the first time that AR expression is diminished in spinal motor neurons early in the disease course of multiple mouse models of ALS, resembling AR inactivation in SBMA. We therefore propose that AR reduction in spinal motor neurons may render them susceptible in ALS and furthermore, ALS and SBMA may share a common disease pathway mediated by disruption of AR expression and trophic signalling.

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P234 HFE H63D MODIFIES DISEASE PATHOPHYSIOLOGY IN AN ALS MOUSE MODEL

NANDAR W, NEELY E, SIMMONS Z, CONNOR J

Penn State University, College of Medicine, Hershey, PA, USA

Email address for correspondence: wnandar@hmc.psu.edu

Keywords: HFE H63D, SOD1^{G93A}, oxidative stress

Background: The HFE H63D gene variant is present in as many as 30% of individuals with amyotrophic lateral sclerosis (ALS). Despite increasing evidence suggesting the association of HFE H63D with ALS, how HFE H63D influences disease processes in ALS remains unclear. HFE H63D is associated with disease processes implicated in ALS such as iron dyshomeostasis and oxidative stress. Thus, HFE H63D is proposed to be a genetic modifier for the risk of ALS.

Objectives: To determine how HFE H63D impacts ALS pathogenesis, we generated a double transgenic mouse line (SOD1/H67D) carrying the HFE H67D (homologue of human H63D) and SOD1^{G93A} mutations.

Methods: We crossed mice carrying HFE H67D with SOD1^{G93A} mice to generate double transgenic mice (SOD1/H67D). A gripstrength meter was used to measure forelimb and hindlimb strength as markers for disease progression. End-stage was defined as the inability of the animal to right itself within 30 s after being placed on its side. Disease duration was the mean time from onset to end-stage. To determine mechanisms by which HFE H63D contributes to ALS pathogenesis, we examined a number of parameters in the lumbar spinal cord of double transgenic mice at 90 days (presymptomatic), 110 days (symptomatic) and end-stage by immunoblot and immunohistochemical analyses.

Results: The double transgenic mice have shorter survival and accelerated disease progression. Transferrin receptor and L-ferritin expression, both indicators of iron status, were altered in double transgenic and SOD1 mice starting at 90 days, indicating iron dyshomeostasis in these mice. However, double transgenic mice had higher L-ferritin expression than SOD1 mice suggesting higher iron in double transgenic mice. In addition to increased L-ferritin, double transgenic mice exhibited increased Iba-1 immunoreactivity and caspase-3 levels, indicating increased microglial activation. Although both SOD1 and double transgenic mice had increased GFAP expression, the magnitude of the increase was higher in double transgenic mice at 110 days, suggesting increased gliosis in these mice. Increased hemeoxygenase-1 and decreased nuclear factor E2-related factor 2 levels in double transgenic mice strongly suggest the accelerated disease process could be associated with increased oxidative stress. There was no evidence of TDP-3 mislocalization to the cytoplasm in double transgenic mice. However, there was evidence suggesting neurofilament disruption in double transgenic mice that has been reported in ALS.

Discussion and conclusion: Our findings indicate HFE H63D modifies ALS pathophysiology via pathways involving oxidative stress, gliosis and disruption of cellular functions. Thus, we hypothesize that HFE H63D increases the risk of ALS by promoting the convergence of disease processes implicated in ALS.

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P235 NEW MODEL OF UBIQUILIN2-RELATED ALS USING AAV VECTORS

BIFERI MG[1], BOS C[1], TANGUY Y[1], ALONSO-MARTIN S[1], ARTEGIANI B[2], RODA M[1], COHEN-TANNOUDJI M[1], FERRY A[1], CAMERINI S[3], CRESCENZI M[3], CALEGARI F[2], RELAIX F[1], BARKATS M[1]

[1]*Center of Research in Myology, Inserm-CNRS-UPMC-UMR 974-Faculté de Médecine, Paris, France,* [2]*DFG-Research Center and Cluster of Excellence for Regenerative Therapies, Dresden, Germany,* [3]*Dept. of Cell Biology and Neurosciences Higher Institute of Health (ISS), Rome, Italy*

Email address for correspondence: mg.biferi@institut-myologie.org

Keywords: mouse model, ubiquilin 2, adeno-associated virus (AAV)

Background: Ubiquilin 2 is a protein involved in degradation pathways via the ubiquitin proteasome system and autophagy. Mutations in Ubiquilin 2 have been identified in inherited forms of ALS or ALS-FTD and represent the first reported mutations in a protein that is directly linked to the protein degradation pathways (1). Ubiquilin 2-positive inclusions have been observed in the spinal cord and brain of both familial and sporadic ALS patients (1), but the pathogenic role of this protein is still unclear. The generation of animal models of the disease is a prerequisite for the understanding of physiopathological mechanisms and identification of new therapeutic strategies for ALS. However, no animal model recapitulating all Ubiquilin 2-related ALS features has been achieved to date using classical transgenesis.

Objectives: According to the evidence suggesting a toxic gain of function of the mutated Ubiquilin 2, we developed a new animal model of Ubiquilin 2-ALS using recombinant adeno-associated-virus (AAV) to overexpress either the wild-type (AAV-UbiWT) or mutant (AAV-UbiPro497His) human Ubiquilin2.

Methods: The vectors were delivered through intracerebroventricular (ICV) injection in newborn FVB mice. An AAV encoding the green fluorescent protein (GFP), injected under the same conditions, was used as control.

Results: The expression of human Ubiquilin2 in brain and spinal cord extracts of infected animals was first evidenced by western blot analysis, one month after injection. The presence of Ubiquilin2-positive inclusions was further detected in the brain and the spinal cord of UbiPro497His overexpressing animals, similarly to ALS-FTD patients. Moreover, the injected animals displayed a reduced brain size compared to controls and a severe astrogliosis in both the spinal cord and the brain. Interestingly, the number of ChAT+ motor neurons was also significantly decreased in the whole spinal cord. Importantly, the AAV-UbiPro497His injected mice had a shortened lifespan (50% of survival at 80 days) and presented a body weight loss phenotype. Mice overexpressing UbiPro497His also developed neurological phenotype with clasping, abnormal spinning and tremors. Muscle weakness, with loss of muscle mass and decrease of muscle strength was observed. The AAV-UbiPro497His-injected mice also displayed enhanced anxiety-related behaviour, one of the first signs of dementia. Overexpression of wild-type Ubiquilin also led to pathological phenotype (albeit to a lesser extent than the one generated by the mutant form), suggesting a crucial role of Ubiquilin 2 pathways also in sporadic ALS forms.

Discussion and conclusion: In conclusion, we have generated the first mouse model of Ubiquilin 2-ALS-FTD displaying most of the clinical and histological features of the human disease. This innovative and successful application of AAV for ALS modelling will be useful to further dissect the molecular mechanisms of this complex pathology and to envision therapeutic strategies.

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P236 HMG-COA REDUCTASE INHIBITORS AND HFE POLYMORPHISM ACCELERATE DISEASE PROGRESSION AND SHORTEN SURVIVAL IN THE SOD1^{G93A} ALS MOUSE MODEL

SU X, NANDAR W, NEELY E, SIMMONS Z, CONNOR J

Penn State College of Medicine, Hershey, PA, USA

Email address for correspondence: xiaoweisu@hmc.psu.edu

Keywords: HMG-CoA reductase inhibitor, HFE polymorphism, coenzyme Q10

Background: Both HMG-CoA reductase inhibitors (statins) and the H63D polymorphism in the HFE iron regulatory gene may impact ALS risk or disease progression. Mitochondrial dysfunction contributes to ALS, and statins perturb mitochondrial enzymes.

Objectives: To determine if statins accelerate disease progression or decrease survival in ALS mouse models; if HFE genotype influences these effects; and if the effects are mediated via statin-induced mitochondrial dysfunction.

Methods: Double transgenic mice harboring SOD1^{G93A} and HFE H67D (homologous to human HFE H63D); single transgenic SOD1 or HFE H67D mice; or wild type (WT) mice, balanced for gender, were used in accordance with IACUC guidelines. For the survival study, 2 mg/kg simvastatin or vehicle was administered daily from disease onset, as determined by rotarod, animals were allowed to reach endstage, defined as the inability to right themselves within 30 seconds. Disease progression was measured by gripstrength. Plasma cholesterol and ferritin levels were measured by colorimetric assay or ELISA. Western blots of mitochondrial fractions from gastrocnemius muscle and lumbar spine were performed. For the mechanism study, animals were administered simvastatin in a similar fashion, gastrocnemius muscle and lumbar spine were collected at the symptomatic 120-day timepoint, and Western blots were performed as above. For the rescue study, 2 mg/kg simvastatin, 10 mg/kg coenzyme Q10, or both, was administered daily from disease onset, animals were allowed to reach endstage. There were 8–13 animals per group. SAS 9.3 or NCSS 9 was used for statistical analyses.

Results: SOD1 mice had increased plasma ferritin levels compared to WT mice. Simvastatin administration and HFE H67D accelerated disease progression as measured by gripstrength. Cox proportional hazards analysis indicated simvastatin administration adversely impacted survival, whereas HFE H67D had a benefit. Coenzyme Q10 administration did not rescue the statin-induced decrease in survival in SOD1 or double transgenic mice. At 120 days and endstage, SOD1 mutant mice had significantly decreased levels of complexes I and IV of the electron transport chain, cytochrome c, and the VDAC1 mitochondrial anion channel in gastrocnemius muscle and lumbar spine compared to WT mice. However, statins did not alter levels of mitochondrial proteins, at 120 days or endstage, in lumbar spine or gastrocnemius muscle.

Discussion and conclusion: Statins accelerate disease progression and decrease survival in SOD1 mutant mice. HFE H67D worsens the statin effect on disease progression while paradoxically benefiting survival. Mitochondrial dysfunction does not mediate these effects. These results suggest patients with ALS receiving statins, especially those harbouring HFE H67D, should be monitored for changes in disease progression. Studies of the effects of statins on disease trajectory in patients with ALS harbouring H63D versus WT HFE may guide clinician use of statins in patients with ALS.

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P237 INVOLVEMENT OF MONOCARBOXYLATE TRANSPORTER 1 IN SOD1^{G93A} DISEASE PATHOGENESIS

PHILIPS T[1], HUGHES EG[2], LEE Y[1], MORRISON BM[1], SATTLER R[1], BERGLES DE[2], ROTHSTEIN JD[1,2]

[1]*Department of Neurology, Brain Science Institute, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA,* [2]*The Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA*

Email address for correspondence: tphilip6@jhmi.edu

Keywords: oligodendrocytes, metabolism, SOD1^{G93A}

Background: In the CNS, oligodendroglia are well established as being involved in myelination of axons and providing rapid saltatory conduction of action potentials. Besides their role in myelination, new studies have provided strong evidence that oligodendrocytes are involved in metabolic support of neurons through the expression of a particular monocarboxylate transporter (MCT) MCT1, which catalyzes the proton linked transport of lactate, ketone bodies and pyruvate across plasma membranes. In the spinal cord of human amyotrophic lateral sclerosis (ALS) patients and SOD1^{G93A} ALS mice, we previously found that MCT1 expression was significantly reduced. The loss of MCT1 mediated trophic support has

been shown to impair neuron and axon viability this might contribute to the selective death of interneurons and motor neurons. In the SOD1^{G93A} mouse decreased MCT1 expression is concomitant with the death and degeneration of oligodendrocytes, and with the failure of newly generating oligodendrocytes to fully mature.

Objectives: The current study is aimed to gain further insight into the involvement of MCT1 transporters during the disease pathogenesis of SOD1^{G93A} mice.

Methods: Using MCT1tdTomato transgenic reporter mice crossbred with SOD1^{G93A} mice, we will explore the percentage of oligodendrocytes with active MCT1 reporter expression at different disease stages. Using PDGFRaRCreER mice crossbred with RosaYFP mice, MCT1tdTomato mice and SOD1^{G93A} mice, we will explore the ability of oligodendrocyte progenitor cells to generate MCT1 reporter expressing oligodendrocytes at both early and late stages of disease.

Results: We found that early disease stage SOD1^{G93A} mice (P60) and WT littermates at all ages analyzed (up to P150) have about 90% of oligodendrocytes, as assessed by CC1 expression, and show MCT1 reporter expression. At symptomatic disease stages in SOD1^{G93A} mice, around 50% of oligodendrocytes had maintained active reporter expression. Fate-map analysis of oligodendrocyte progenitor cells differentiating into CC1 oligodendrocytes demonstrated that at all disease stages at least some oligodendrocyte progenitor cells were still able to generate MCT1 reporter expressing oligodendrocytes, but MCT1 reporter expression was only initiated late during the differentiation, well after initiation of MBP protein expression. At these later disease stages, many oligodendrocytes were immature and had not initiated MCT1 reporter expression yet, and as such failed to provide trophic support to motor neurons.

Discussion and conclusion: In SOD1^{G93A} mice, many oligodendrocytes die and are replaced with newly generated oligodendrocytes generated form oligodendrocyte progenitor cells. At later disease stages, despite the ability of newly generated oligodendrocytes to turn on MCT1 reporter expression, many of these cells had not matured fully and failed to provide trophic support to motor neurons. We are currently trying to modulate MCT1 expression in oligodendrocytes and explore whether this affects the oligodendrocyte ability to provide trophic support to neurons.

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P238 HSP110 AS A MODIFIER OF TOXICITY IN A MOUSE MODEL OF SOD1-LINKED ALS

FENTON WA[1], NAGY M[1,2], LI D[1,2], FURTAK K[1,2], HORWICH AL[1,2]

[1]*Yale University School of Medicine, New Haven, CT, USA,* [2]*Howard Hughes Medical Institute, New Haven, CT, USA*

Email address for correspondence: wayne.fenton@yale.edu

Keywords: molecular chaperone, HSP110, SOD1^{G85R}

Background: A number of recent studies have suggested that Hsp110 chaperones can collaborate with Hsp70s to forestall or reverse protein aggregation. In an earlier study, we observed that all three mouse Hsp110s were recovered in association with affinity-captured SOD1^{G85R} -YFP from the spinal cord of ALS mice expressing the mutant SOD1. More recently, we showed that human HSPA4L, an Hsp110, could prevent the toxic effect of purified SOD1^{G85R} -YFP on anterograde fast axonal transport in squid giant axon axoplasm, preventing activation of a MAP kinase cascade by the mutant misfolded protein.

Methods: We programmed transgenic mice expressing this chaperone from a Thy1.2 (neuronal) promoter and crossed them with ALS mice expressing SOD1^{G85R} -YFP, a misfolded protein that forms large aggregates in spinal cord motor neurons. The latter animals develop typical signs of lower motor neuron disease and paralysis by 5–6.5 months.

Results: Two independent doubly transgenic lines were evaluated. For one, transgenic Hsp110 mRNA is expressed in brain at 2–2.5 times the level of the corresponding mouse Hsp110. Sixty-five animals of this strain, with Hsp110 copy numbers of ~80–150, have been followed for the past year. We have observed median survival to be increased by 2 months, compared both with littermates lacking Hsp110 (35 mice), none of which survived beyond 6.5 months, and with our parental ALS line. Strikingly, ~30% of the double transgenic animals of this line continue to survive beyond 8 months of age, with several now 11 months old. Sections of the spinal cord of one of the Hsp110 animals at 5 months of age has been examined microscopically, revealing a larger number of surviving motor neurons than typically seen in a pre-end-stage G85R mouse and fewer neurons with large aggregates. Animals from the second line are several months younger, but also appear to be exhibiting extended survival. We are further evaluating these lines by qRT-PCR and LC/MS analysis of motor neuron cell bodies captured by LMD.

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P239 MUSCLE-SPECIFIC CONTROL OF HSP70 IN POLYGLUTAMINE INDUCED MOTOR NEURON DISEASE

KONDO N, KATSUNO M, ADACHI H, SAHASHI K, MIYAZAKI Y, IIDA M, TOHNAI G, ISHIGAKI S, FUJIOKA Y, TANAKA F, SOBUE G

Nagoya University, Nagoya, Japan

Email address for correspondence: naohidek@med.nagoya-u.ac.jp

Keywords: SBMA, heat shock proteins, skeletal muscle

Background: Spinal and bulbar muscular atrophy (SBMA), also called Kennedy's disease, is an adult-onset motor neuron disease caused by the expansion of a CAG repeat within the first exon of the androgen receptor gene. Heat shock proteins (Hsps) such as Hsp70 play a defensive role in the pathophysiology of neurodegenerative disorders by solubilising pathogenic abnormal proteins. Although heat shock factor-1 (Hsf-1) controls the expression levels of Hsps, the molecular basis for tissue specific control of Hsps in motor neuron diseases was not clear.

Methods: To reveal the mechanism of Hsps regulation in the skeletal muscle of SBMA, we studied the skeletal muscle from the model mice of SBMA, in which Hsf-1 is heterozygously knocked out. We performed western blotting and Immunohistochemistry of skeletal muscle from wild-type; AR-97Q (SBMA model: 97Q$^{Tg/-}$, Hsf-1$^{+/+}$); and AR-97Q*Hsf-1*$^{+/-}$ (heterozygous Hsf-1 knockout SBMA model: 97Q$^{Tg/-}$, Hsf-1$^{+/-}$) mice using anti-Hsf-1, anti-Hsp72, anti-Nfya, anti-Sp1, anti-p53, anti-Tbp and anti-polyglutamine antibodies.

Results: On western blot analysis, the expression level of Hsp72, the inducible form of Hsp70, in the spinal cord of SBMA mice was downregulated by heterozygous knock out of Hsf-1. Conversely, Hsp72 expression level in the skeletal muscle of these mice was maintained despite Hsf-1 depletion. Moreover, the depletion of Hsf-1 did not enhance the pathogenic AR accumulations in the skeletal muscle of SBMA mice. Surprisingly, in the skeletal muscle of SBMA mice, Nfya and Sp1 were upregulated compared with wild-type mice both on immunohistochemistry and western blotting. Furthermore, this reaction was prominent in the Hsf-1 depleted SBMA mice. In contrast to the skeletal muscle, neither Nfya nor Sp1 were upregulated by Hsf-1 depletion in the spinal cord of SBMA and heterozygous Hsf-1 knockout SBMA mice.

Conclusion: These results suggest that the regulatory system of Hsps in skeletal muscle is distinct from that in the central nervous system in SBMA.

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P240 ABSENCE OF C5A-C5AR1 SIGNALLING DIMINISHES INFILTRATION OF PERIPHERAL IMMUNE CELLS IN SKELETAL MUSCLE OF HSOD1^{G93A} MICE

WANG H[1], LEE J[1], WOODRUFF T[1], NOAKES P[1,2]

[1]*School of Biomedical Sciences,* [2]*Queensland Brain Institute, The University of Queensland, Queensland, Australia*

Email address for correspondence: haitao.wang@uqconnect.edu.au

Keywords: complement system, C5aR1, peripheral immune cells

Background: The terminal innate immune complement system has recently been implicated in the pathogenesis of amyotrophic lateral sclerosis (ALS). Our previous studies in the hSOD1^{G93A} mouse model of ALS demonstrated excessive complement activation, C5a receptor (C5aR1) microglial upregulation in the lumbar spinal cord (1). Importantly, the absence of C5aR1 in these hSOD1^{G93A} mice reduced disease pathology (2).

Objectives: The present study aimed to determine the expression of complement components (C1qB, C3 and C5aR1) at both mRNA and protein levels in tibialis anterior (fast-twitch) and soleus (slow-twitch) muscles of hSOD1^{G93A} mice. Furthermore, we investigated the role of C5a-C5aR1 signalling in the infiltration of peripheral immune cells (macrophages and helper T lymphocytes) in tibialis anterior (TA) and soleus (SOL) muscles of hSOD1^{G93A} mice.

Methods: TA and SOL muscles from hSOD1^{G93A}, hSOD1^{G93A} lacking C5aR1 (hSOD1^{G93A}×C5aR1$^{-/-}$) and wild-type (WT) mice were examined at 3 different stages of disease progression. The mRNA level of complement factors C1qB, C3 and C5aR1 were measured in WT and hSOD1^{G93A} mice using quantitative real-time PCR. Protein expression level of C5aR1 was also examined using western blotting. Cellular localisation of C5aR1 was investigated using immunohistochemistry with combinations of antibodies specific for neuromuscular junctions (α-Bungaratoxin), Schwann cells (S100), macrophages (CD11b) and helper T lymphocytes (CD4). The number of macrophages and helper T lymphocytes were also counted in TA and SOL muscles of WT, hSOD1^{G93A} and hSOD1^{G93A}×C5aR1$^{-/-}$ mice.

Results: We found elevated levels of C1qB, C3 and C5aR1 in TA and SOL muscles of hSOD1^{G93A} mice during disease progression. Immuno-localisation showed that C5aR1 was expressed predominantly on the macrophages in WT and hSOD1^{G93A} mice. Furthermore, we demonstrated a significant increase of macrophage and helper T lymphocyte numbers in TA and SOL muscles of hSOD1^{G93A} mice when compared to WT mice during disease progression. The infiltration of immune cells in TA muscle was far greater when compared to SOL muscle in hSOD1^{G93A} mice. Interestingly, hSOD1^{G93A}xC5aR1$^{-/-}$ mice showed decreased numbers of macrophages and helper T lymphocytes when compared to hSOD1^{G93A} mice.

Discussion and conclusion: These results indicate that complement activation occurs in the muscle tissue of hSOD1^{G93A} mice. In addition, increased C5a-C5aR1 signalling may contribute to the recruitment of peripheral immune cells that may accelerate muscle denervation. The amounts of infiltrating immune cells may also reflect the degree of muscle denervation as more immune cells were observed in TA, a fast-twitch muscle that is more vulnerable to degeneration in ALS.

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P241 INDUCTION OF IMMUNOPROTEASOME AND MAJOR HISTOCOMPATIBILITY COMPLEX I (MHCI) IN MOTOR NEURONS OF A FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS (ALS) MOUSE MODEL

NARDO G, TROLESE MC, BOSANI B, ROSSI M, BENDOTTI C

IRCCS - Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy

Email address for correspondence: giovanni.nardo@marionegri.it

Keywords: immune system, MHCI, transgenic mouse model

Background: We recently found, in the spinal MN of SOD1^{G93A} mice a significant upregulation of the immunoproteasome subunit, the large multifunctional peptidase 7 (LMP7), which starts at the presymptomatic stage and progressively increased during the disease progression (1, 2). Immunoproteasome recognizes and degrades polyubiquitinated protein substrates to generate small protein fragments that can be used by major histocompatibility complex (MHC) class I molecules for the display of antigens to CD8+T cells (3).

Objectives: We aimed to examine the expression of MHCI pathway at the central and peripheral nervous system of a familial ALS mouse model.

Methods: An extensive immunohistochemical and confocal microscopy analysis has been performed in the spinal cord, peripheral nerves and muscles of SOD1^{G93A} mice during the disease course and the associated β2 microglobulin (β2m), together with LMP7.

Results: We report that MN and surrounding glial cells (microglia, olygodendrocytes but not astrocytes) exhibit the activation of LMP7, MHC-I and β2m at very early stages of the disease. Notably, while the immunostaining of LMP7 and β2m were highly increased in the perikarya of MNs and motor axons, the MHC-I immunoreactivity was increased exclusively in the motor axons and neuromuscular junction (NMJ) of SOD1^{G93A} mice during the disease course. Consistently, we found CD8+T lymphocytes infiltrates in the spinal cord, sciatic nerve and muscle of SOD1^{G93A} as demonstrated by CD3 positive immunoreactivity and real time PCR, suggesting the interaction of MNs with cytotoxic T cells through MHCI.

Discussion and conclusion: These data point out that the activation of the adaptive immune system molecules both at central and peripheral level may take part in the pathogenesis and / or progression of ALS. Studies are ongoing to investigate the beneficial or detrimental effect of this immune response in SOD1^{G93A} mice.

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P242 BENEFICIAL EFFECTS OF A SYNTHETIC PROSTAGLANDIN I2 AGONIST, ONO1301, IN ANIMAL MODELS OF ALS

TADA S[1,2], OKUNO T[3], YASUI T[1], NAKATSUJI Y[3], SAKAI Y[4], MIYAGAWA S[4], SAWA Y[4], HAZAMA T[2], KIKUTANI H[1], SAKODA S[5], MOCHIZUKI H[3]

[1]*Department of Molecular Immunology, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan,* [2]*Department of Neurology, Osaka General Medical Center, Osaka, Japan,* [3]*Department of Neurology,* [4]*Department of Cardiovascular Surgery, Osaka University Graduate School of Medicine, Osaka, Japan,* [5]*National Toneyama Hospital, Osaka, Japan*

Email address for correspondence: tada@neurol.med.osaka-u.ac.jp

Keywords: ONO1301, vasodilation therapy, HIF1 alpha

Background: Accumulating studies suggest that there is a link between altered vascular structure, reduced blood flow in the spinal cord and neurodegeneration in animal models of ALS (1, 2). However, the effect of vasodilation therapy with a prostaglandin I2 agonist, which is an effective vasodilator, against ALS *in vivo* remains totally unknown.

Objectives: Our objective here is to explore the role of blood flow increase in neurodegeneration of ALS by using ONO1301, a novel synthetic prostaglandin I2 agonist with a vasodilator effect.

Methods: We prepared a novel sustained-release prostaglandin I2 agonist polymerized with poly (D,L-lactic-co-glycolic acid) (PLGA) microspheres to realize long-lasting effects (ONO1301MS). We treated mice carrying the human SOD-1 mutation G93A (mSOD1^{G93A} mice) with ONO1301MS (n = 14) or vehicle (n = 13), and assessed neurodegeneration by measuring body weight, motor function and survival time. We assessed the expression of several genes associated with hypoxia in the spinal cord of ONO1301MS or vehicle treated mSOD1^{G93A} mice with quantitative RT-PCR. We also conducted immunohistochemistry to show the different expression levels of hypoxia inducible factor 1 (HIF1alpha) in the spinal cords of mSOD1^{G93A} mice.

Results: ONO1301MS significantly improved motor function of mSOD1^{G93A} mice at 17, 19 and 20 weeks of age although it did not affect body weight and survival time of them. To confirm the vasodilation effect of ONO1301MS *in vivo*, we conducted immunohistochemical analysis of the spinal cord of mSOD1^{G93A} mice. ONO1301MS significantly decreased the expression level of HIF1 alpha, suggesting that it had increased the blood flow and ameliorated the hypoxia the spinal cord of mSOD1^{G93A} mice.

Discussion and conclusion: Our study showed that a synthetic prostacyclin agonist, ONO1301, had beneficial effects against neurodegeneration in mSOD1^{G93A} mice. The favourable effects could be attributable to the increased blood flow and amelioration of hypoxia in the spinal cord of mSOD1^{G93A} mice. Further study is necessary to fully characterize the neuroprotective action of ONO1301. We might consider other vasodilation agents as therapeutic potentials for the treatment of ALS.

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P243 THE ROLE OF EPHRIN-B2 IN AMYOTROPHIC LATERAL SCLEROSIS

SCHOONAERT L[1,2], RUÉ L[1,2], POPPE L[1,2], TIMMERS M[1,2], VAN HOECKE A[4], VAN DAMME P[2,3], LEMMENS R[2,3], ROBBERECHT W[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]*University Hospitals Leuven, Department of Neurology, Leuven, Belgium,* [4]*Max-Planck-Institute of Neurobiology, München, Germany*

Email address for correspondence: Lies.Schoonaert@vib-kuleuven.be

Keywords: ephrin-B2, astrocyte, EphA4

Background: Amyotrophic lateral sclerosis (ALS) is characterized by considerable genetic heterogeneity since mutations in more than 10 different genes (eg, SOD1, FUS, TDP, C9ORF72) are known to cause the hereditary form of ALS. Similar heterogeneity is observed in the clinical presentation. This indicates that there are factors that modify the phenotypic expression of the disease. The tyrosine kinase receptor EphA4 was recently shown to be a modifier of ALS. Genetic and pharmacological inhibition of EphA4 rescued the phenotype in a zebrafish model of ALS and increased survival in

ALS rodent models. In ALS patients an inverse correlation was found between EphA4 expression and disease onset. However, the mechanism of action has not yet been fully elucidated. EphA4 interacts with ephrin-a and ephrin-b ligands. Several of these EphA4 interaction partners have been shown to be not only expressed on motor neurons, but also on astrocytes, microglia and oligodendrocytes. These cells surrounding the motor neurons play an important role in the pathogenesis of ALS. Here, we aimed to determine the contribution of these various cell types and one specific EphA4 ligand, ephrin-b2, in ALS disease progression.

Methods: First we performed immunofluorescence staining of ephrin-b2 in the spinal cord of an ALS mouse model, overexpressing mutant SOD1 ($SOD1^{G93A}$) and compared this pattern to mice overexpressing wild-type SOD1 ($SOD1^{WT}$) at different stages of the disease.

Results: In $SOD1^{WT}$ spinal cord we observed ephrin-b2 to be highly expressed in motor neurons and oligodendrocytes, while only faint expression was detected in astrocytes. In symptomatic $SOD1^{G93A}$ spinal cord the expression pattern of ephrin-b2 in astrocytes and motor neurons changed. Immunoreactivity was markedly upregulated in astrocytes, but the presence of ephrin-b2 was clearly reduced in the neuronal population. As the expression pattern changed in the different cell types with disease progression, we next explored a possible modifying cell-specific role of ephrin-b2 in ALS, by generating a conditional ephrin-b2 knockout mouse, in which ephrin-b2 is deleted upon GFAP expression. Deleting ephrin-b2 in reactive astrocytes of the $SOD1^{G93A}$ ALS mouse model resulted in a delay of disease onset and prolonged disease duration. These results suggest astrocytic ephrin-b2 to play a role in modifying ALS.

Discussion and conclusion: In future experiments we intend to further explore the cellular mechanism of ephrin signalling in the pathophysiology of motor neurodegeneration.

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P244 INCREASED OREXIN PROMOTES SLEEP/WAKE DISTURBANCES AND INTERACTS WITH SIRT1 IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

LIU R, CAI B, FAN D

Peking University Third Hospital, Beijing, China

Email address for correspondence: dsfan2010@aliyun.com

Keywords: sleep/wake disturbance, orexin, SIRT1

Objective: Sleep/wake disturbances of amyotrophic lateral sclerosis (ALS) patients are well documented, whereas corresponding animal or mechanistic study on sleep disturbances in ALS are lacking Orexin and SIRT1 are important molecules in sleep/wake regulation.

Methods: In this study, we used $SOD1^{G93A}$ transgenic mice as ALS mouse model. EEG/EMG recordings, quantitative reverse transcriptase PCR, western blot, ELISA, co-immunoprecipitation and immunofluorescence were performed between $SOD1^{G93A}$ transgenic mice and their littermate control mice at the age of 90 days and 120 days.

Results: In $SOD1^{G93A}$ transgenic mice, for the first time, we observed significantly enhanced wakefulness time, reduced sleep time and up-regulated orexins (prepro-orexin, orexin A and B). SIRT1 is also increased in symptomatic $SOD1^{G93A}$ transgenic mice and interacts with prepro-orexin in hypothalamus of $SOD1^{G93A}$ transgenic mice.

Discussion and conclusion: Increased orexins promote wakefulness, result in sleep/wake disturbances and interact with SIRT1 in the hypothalamus in ALS mouse model. This interaction could prevent SIRT1 from neuroprotective effects in ALS and contribute to the disease process. Therefore, sleep disturbances are not only symptoms that occur early in ALS, but also factors that promote ALS progression. This could be common mechanisms in all neurodegenerative diseases, and orexins/SIRT1 pathways might be potential target to retard the disease process in the early stages.

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P245 GOLGI FRAGMENTATION IN PMN MICE IS DUE TO A DEFECTIVE ARF1/TBCE CROSS TALK THAT COORDINATES COPI VESICLE FORMATION AND TUBULIN POLYMERIZATION

BELLOUZE S[1], SCHAEFER M[2], BUTTIGIEG D[1], BAILLAT G[1], RABOUILLE C[3], HAASE G[1]

[1]*CNRS UMR7289 and Aix-Marseille University, Marseille, France,* [2]*Klinik für Anaesthesiologie, Universität Mainz, Mainz, Germany,* [3]*Hubrecht Institute-KNAW and University Medical Center Utrecht and The Department of Cell Biology, Utrecht, The Netherlands*

Email address for correspondence: georg.haase@univ-amu.fr

Keywords: Golgi fragmentation, microtubules, vesicle trafficking

Background: Golgi fragmentation is an early hallmark of amyotrophic lateral sclerosis (ALS) and other neurodegenerative diseases affecting motor neurons. Yet, its pathophysiological relevance and molecular mechanisms are unclear.

Objectives: To better understand the mechanisms of Golgi fragmentation, we investigated a model of human motor neuron disease, progressive motor neuronopathy *(pmn)* mice mutated in the Golgi-localized tubulin-binding cofactor E (TBCE), together with TBCE-depleted motor neuron cultures.

Results: We demonstrate severe and progressive Golgi fragmentation in motor neurons of *pmn* mice. Loss of TBCE function in mutant *pmn* and TBCE-depleted motor neuron cultures causes defects in Golgi-derived microtubules, as expected, but surprisingly also reduced levels of the COPI subunits β-COP and ε-COP, decreased recruitment of the Golgi tethering factors p115/GM130 and impaired vesicle fusion mediated by the Golgi SNAREs GS15/GS28. Conversely, the small GTPase ARF1, which stimulates COPI vesicle formation, enhances the recruitment of TBCE to the Golgi, increases polymerization of Golgi-derived microtubules and rescues TBCE-linked Golgi fragmentation. Importantly, transgenic mutant SOD1 mice display a similar Golgi fragmentation and dys-regulation of COPI subunits, Golgi tethering factors and Golgi SNAREs as *pmn* mice.

Discussion and conclusion: Our data unravel a novel ARF1/TBCE-mediated cross talk that coordinates COPI vesicle formation and tubulin polymerization at the Golgi apparatus. Interruption of the ARF1/TBCE cross talk causes Golgi fragmentation in *pmn* mice and possibly also in human SOD1-linked ALS.

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P246 ROLE OF CYSTEINE RESIDUE OF MUTANT SOD1 IN THE PATHOGENESIS OF ALS

NAGANO S[1], YAMAMOTO K[1], URUSHITANI M[2], FUJIWARA N[3], ARAKI T[1]

[1]*National Institute of Neuroscience, National Center of Neurology and Psychiatry, Kodaira, Tokyo, Japan,* [2]*Department of Neurology, Kyoto University Graduate School of Medicine, Kyoto, Kyoto, Japan,* [3]*Department of Biochemistry, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan*

Email address for correspondence: nagano@ncnp.go.jp

Keywords: SOD1, cysteine, oxidation

Background: Previously we have reported that the cysteine residue (Cys111) near the dimer contact site is critical to generate monomers and aggregates of mutant Cu Zn-superoxide dismutase (SOD1) protein, which is thought to be toxic to motor neurons in familial ALS. However, it is not ascertained whether the residue contributes to the motor-neuronal toxicity of mutant SOD1 *in vivo*.

Objectives: To verify the significance of Cys111 in the pathogenesis of ALS, we aimed to analyze the phenotype of mutant SOD1 transgenic mice in relation to the existence of Cys111.

Methods: We generated SOD1 transgenic mice harbouring H46R mutation alone or H46R plus C111S mutations. Motor performance test and pathological/biochemical analyses of the spinal cord were done to compare the toxicity of mutant SOD1 to motor neurons in each mouse line.

Results: The onset of the disease was delayed and the survival time was extended in $SOD1^{H46R/C111S}$ mice compared with $SOD1^{H46R}$ mice. Motor neuron loss and astrocyte/microglia activation was trivial in the spinal cord of $SOD1^{H46R/C111S}$ mice at the time H46R mice reached to the endpoint. Misfolded or insolubly aggregated SOD1 was seen in the spinal cord of H46R mice at the endpoint, whereas such SOD1 species was not detected in age-matched H46R/C111S mice. Cys111-oxidized SOD1 appeared in the spinal cord of H46R mice throughout their life, which was more prominent than that in wild type SOD1 transgenic mice.

Discussion and conclusions: It was suggested that Cys111 of mutant SOD1 have a key role in the appearance of ALS by generating oxidation-mediated monomerization and aggregation of the protein. Cys111 is thought to take part in the first step to change the high-order structure than the aggregation process of mutant SOD1. The blockage of oxidative modification of Cys111 in mutant SOD1 will be expected as a novel treatment strategy of ALS.

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P247 GENDER SPECIFIC BENEFICIAL EFFECTS OF DOCOSAHEXAENOIC ACID DIETARY SUPPLEMENTATION IN ALS MICE

TORRES P[1], CACABELOS D[1], AYALA V[1], BOADA J[1], CABRÉ R[1], NAUDÍ A[1], POVEDANO MP[2], PAMPLONA R[1], PORTERO-OTIN M[1]

[1]*IRBLLEIDA-UdL, Lleida, Catalonia, Spain,* [2]*Hospital de Bellvitge, Barcelona, Catalonia, Spain*

Email address for correspondence: jboada@mex.udl.cat

Keywords: dietary intervention, fatty acid metabolism, $SOD1^{G93A}$

Background: Although the underlying causes of motor neuron degeneration in ALS remain largely unknown, docosahexaenoic acid (DHA), a key fatty acid in nervous system homeostasis, is depleted in spinal cord post-mortem samples of ALS patients, confirming the contribution of changes in fatty acid metabolism to the pathogenesis of ALS (1). A gender bias exists in ALS, with higher incidence and prevalence in men than in women, and different clinical phenotypes, which is also observed in animal models of ALS (2).

Objectives: In this study we evaluated the effect of dietary changes (n-3 fatty acid depletion with and without DHA supplementation) aimed to modify tissue fatty acid composition (3) on survival, disease onset and inflammatory and oxidative stress markers in male and female $hSOD1^{G93A}$ transgenic mice.

Methods: Survival was assessed by Kaplan-Meier analysis. Spinal cord fatty acid profiles were determined after whole lipid extraction, hydrolysis and gas chromatography analysis of their methyl esters.

Results: Male ALS mice survival was extended ($p < 0.0001$; $n = 14$) under DHA dietary supplementation and equalled female lifespan, without modifying the age of disease onset, whereas n-3 fatty acid depletion had no effect in both genders. DHA supplementation resulted in an increase in DHA content at p60 (pre-symptomatic stage) and p90 (disease onset), whereas the peroxidability index was diminished at endpoint. The content of arachidonic acid, a precursor of proinflammatory mediators, and the derived anti-inflammatory index decreased at the three time points. These changes in fatty acid profiles and derived indexes were similar in both genders. On the other hand, n-3 PUFA depletion did not provoke marked changes in the fatty acid profiles. In line with DHA content increase, WB analyses showed that DHA supplementation increased levels of syntaxin 3-DHA dependent synaptic protein in spinal cord of male, but not female, ALS mice.

Discussion and conclusion: Our results demonstrate that DHA supplementation in diets during the pre-symptomatic stage extends survival in male but not female ALS mice, concomitant with an increase in spinal cord DHA content until disease onset, but not at endpoint. These results reinforce the role of gender as a relevant factor in the design of dietary interventions in ALS patients, and suggest a greater beneficial effect of early DHA supplementation in male ALS patients.

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P248 NEUROPROTECTIVE EFFECTS OF A CELL-FREE EXTRACT DERIVED FROM HUMAN ADIPOSE STEM CELLS IN AMYOTROPHIC LATERAL SCLEROSIS

PARK KH[1], JEON GS[1], SOHN SY[1], SUNG J-J[1], OH J-H[2]

[1]*Seoul National University Hospital, Seoul, Republic of Korea,* [2]*Jeju National University Hospital*

Email address for correspondence: gyesun@daum.net

Keywords: SOD1, apoptosis, human adipose stem cells

Background: Amyotrophic lateral sclerosis (ALS) is a devastating human neurodegenerative disease. The aetiology and pathogenic mechanisms of the disease remain unknown, and there is no effective treatment. Human adipose stem cells (hASC) are an easily available source of stem cells. Since hASC can be differentiated into neuronal stem cells, they have clinically feasible potential for neurodegenerative disease.

Objectives: The cytosolic extracts of hASC contain a number of neurotrophic factors. Here, we investigated effects of the hASC extract on $SOD1^{G93A}$ mouse ALS model and motor neuronal cells (NSC-34).

Results: The hASC extract administration improved motor function and prolonged the time until symptom onset, rotarod failure, and death in transgenic mice with ALS compared to control mice. NSC-34 cells treated with the hASC extract showed decreased mutant SOD1-induced cell toxicity and cell apoptosis.

Conclusion: These results suggest that the hASC is promising as a novel therapeutic strategy for ALS.

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P249 REDUCING THE EXPRESSION OF MGLU1 AND MGLU5 RECEPTORS AMELIORATES SURVIVAL AND DISEASE PROGRESSION IN $SOD1^{G93A}$ MICE

MILANESE M[1], BONIFACINO T[1], MELONE M[2], GIRIBALDI F[1], MUSANTE I[3], PULITI A[3,4], CONTI F[2], BONANNO G[1,4]

[1]*Department of Pharmacy, Pharmacology and Toxicology Unit,* [4]*Center of Excellence for Biomedical Research, University of Genoa, Genoa, Italy,* [2]*Department of Experimental and Clinical Medicine, Unit of Neuroscience and Cell Biology, Università Politecnica delle Marche and Center for Neurobiology of Aging, INRCA IRCCS, Ancona, Italy,* [3]*Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics and Maternal and Child Health, Medical Genetics Unit, University of Genoa and Gaslini Institute, Genoa, Italy*

Email address for correspondence: milanese@pharmatox.unige.it

Keywords: Group I metabotrophic glutamate receptors, glutamate-mediated excitotoxicity, $SOD1^{G93A}$

Background: Glutamate (Glu)-mediated excitotoxicity plays a major role in the degeneration of motor neurons (MNs) in amyotrophic lateral sclerosis (ALS). Besides the impaired glial glutamate transport (1), an excessive Glu release has been also found in the spinal cord of experimental ALS mice (2). Our recent pharmacological studies suggest that the hyper-activation of Group I metabotropic Glu receptors (mGluR1 and mGluR5), expressed at pre-synaptic level in the spinal cord of ALS mice, play a role in this scenario (3).

Objectives: To investigate the effect of the mGluR1 or mGluR5 down-regulation in ALS.

Methods: To provide a genetic tool to evaluate the role of mGluR1 and mGluR5 in ALS, we generated mice carrying half expression of mGluR1 in the $SOD1^{G93A}$ background, by crossing $SOD1^{G93A}$ mice with heterozygous $mGluR1^{+/-}$ mice. In the same line, we also generated mice carrying half expression of mGluR5, by crossing $SOD1^{G93A}$ mice with $mGluR5^{+/-}$ mice. Life span, motor abilities, MNs preservation, mitochondrial damage, oxidative stress markers, astrogliosis and microglia activation, receptor expression and glutamate release were investigated to characterize double mutant mice compared to the $SOD1^{G93A}$ ALS model.

Results: The $SOD1^{G93A}mGluR1^{+/-}$ mice showed a delayed pathology onset, improved motor performances and prolonged survival probability, compared to $SOD1^{G93A}$ mice. These results were associated with reduction of spinal cord motoneuron death, decreased astrocyte and microglia activation, down-regulation of oxidative stress markers and reduced mitochondrial damage. As functional results we also registered a normalization of the abnormal glutamate release induced by the activation of mGluR1 and mGluR5 in $SOD1^{G93A}$ $mGluR1^{+/-}$ compared to $SOD1^{G93A}$ mice. Interestingly, knocking-down mGluR1 also reduced mGluR5 spinal cord expression. $SOD1^{G93A}$ $mGluR5^{+/-}$ animals showed a delayed pathology onset and a remarkable prolonged life span, although these data were not accompanied by improved motor performances and phenotype amelioration respect to $SOD1^{G93A}$ mice. Differently from what we observed in $SOD1^{G93A}$ $mGluR1^{+/-}$ mice, reducing mGluR5 does not affect mGluR1 expression.

Discussion and conclusion: Our findings so far demonstrate that mGluR1 or mGluR5 down-regulation has a significant impact *in vivo* on ALS clinical outcome and provide a rationale for pharmacological approaches based on the selective block of Group I mGluRs.

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P250 SYNAPTIC BOUTONS AND DENDRITIC VACUOLIZATION IN ADULT MOTONEURONS OF MSOD1-G93A MOUSE MODEL OF ALS

DELESTRÉE N, MARTINOT C, MANUEL M, ZYTNICKI D

University Paris Descartes, UMR CNRS 8119, Paris, France

Email address for correspondence: nicolas.delestree@gmail.com

Keywords: varicosities, synapses, $SOD1^{G93A}$

Background: A striking pathological feature observed in mSOD1 mouse models of ALS is the presence of vacuoles in the soma, the axon and also the dendrites of their motor neurons (1). The mechanism leading to the vacuolization is not known. However, vacuoles might originate through dilation of the mitochondrial intermembrane space (2).

Objectives: We investigated where the vacuoles appear along the dendritic tree, how they evolve during the time course of the disease and how synaptic boutons are distributed with respect to the dendritic vacuoles.

Methods: Spinal motor neurons were recorded intracellularly and labelled with neurobiotin in anaesthetized mice. Our preparation also allows us to record motor units EMG in response to intracellular stimulation to asses the state of denervation of motoneurons. After intracardiac perfusion of the mouse, spinal sections were processed for immunolabelling of excitatory (VGLUT1 and VGLUT2) and inhibitory (VGAT) boutons. Z-stacks were analysed using Neurolucida software.

Results: Both WT and $SOD1^{G93A}$ mice display swellings along their dendrites (same density: WT 67 ± 28 vs. SOD 65 ± 21 swellings/mm), but vacuolization appears in the dendritic swellings of mSOD1 motoneurons only. This vacuolization process has already started by P40 in motoneurons still able to excite their muscle fibers, indicating that the neuromuscular junctions are still intact. Afterward, dendritic vacuoles dramatically grow throughout the disease to reach diameters as large as 15 micrometers at P110. Swellings are also present in mice expressing non-mutated human SOD1 but at P180 they still do not display vacuolization. The average densities of excitatory (VGLUT1 and VGLUT2) and inhibitory (VGAT) boutons that contact the dendritic tree (measured on 350–1500 µm of dendritic length for each cell) are unchanged at P40–50 in mSOD1 ($n = 6$) mice compared to WT ($n = 6$) mice (VGAT: WT 0.54 ± 0.09 vs. SOD1 0.45 ± 0.08 boutons/µm; VGLUT1: WT 0.06 ± 0.04 vs. SOD1 0.07 ± 0.02 boutons/µm; VGLUT2: WT 0.42 ± 0.10 vs. SOD1 0.49 ± 0.07 boutons/µm). Unexpectedly, we found that VGLUT2 and VGAT boutons tend to cluster on swellings in both WT and mSOD1 dendrites creating hotspots of extensive ion influx and high metabolic activity.

Discussion and conclusion: Our data suggest that there might be a causal link between synaptic activity and dendritic vacuolization in spinal motor neurons of ALS mice. Our work is reinforcing the hypothesis that excitotoxicity can lead to mitochondrial damages and ultimately to degeneration of motor neurons in ALS.

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P251 NEUROPROTECTIVE AND ANTI-NEUROINFLAMMATORY EFFECTS OF MECASIN VIA UP-REGULATION OF HEME OXYGENASE-1 IN MOUSE HIPPOCAMPAL AND MICROGLIAL CELLS

LEE J-C[1], KIM S-C[1], LEE D-S[2], KIM Y-C[3]

[1]*Center of Amyotrophic lateral Sclerosis, Wonkwang University Hospital, Gwangju, Republic of Korea,* [2]*Department of Biochemistry, Inha University School of Medicine, Incheon, Republic of Korea,* [3]*Institute of Pharmaceutical Research and Development, College of Pharmacy, Wonkwang University, Iksan, Jeollabuk-do, Republic of Korea*

Email address for correspondence: ljc1339@hanmail.net

Keywords: anti-glutamate toxicity, neuroprotective, anti-neuroinflammatory

Background: One of the various causes of ALS is known as glutamate toxicity. Oxidative stress and neuroinflammation have also been implicated in many neurodegenerative diseases. In previous studies on microglial responses in central nervous system (CNS) inflammation, microglial activation was induced by a variety of agents, including the bacterial product lipopolysaccharide (LPS), and proinflammatory cytokines such as interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α). Heme oxygenase (HO)-1, an enzyme essential for heme degradation, has been shown to exert antioxidative and anti-inflammatory effects under various conditions.

Objectives: The aim of this study was to evaluate neuroprotective and anti-neuroinflammatory effects of mecasin in mouse hippocampal and microglial cells on glutamate- or LPS-induced neuronal cell injury.

Methods: Mecasin, a combination of natural plant extracts, was obtained from *Curcuma longa, Salvia miltiorrhiza, Gastrodia elata, Chaenomeles sinensis, Polygala tenuifolia, Paenia japonica, Glycyrrhiza uralensis, Atractylodes japonica* and *Aconitum carmichaeli,* and was freshly dissolved in dimethyl sulfoxide. The effects of Mecasin on cell viability were studied using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The levels of prostaglandin E2 (PGE2), TNF-α, and interleukin-1β (IL-1β) were also evaluated. The expression of inducible nitric oxide synthase (iNOS), cyclooxygenase (COX)-2, HO-1, and the nuclear factor-E2-related factor 2 (Nrf2) were evaluated by Western blot using specific antibodies. In addition, the translocation of Nrf2 was also assessed by immunofluorescence microscopy.

Results: At the non-cytotoxic concentrations, Mecasin (10, 50, 100, 200 µM) increased the cellular resistance of HT22 cells to oxidative injury caused by the glutamate-induced cytotoxicity by nuclear translocation of Nrf2-mediated HO-1 expression in a concentration-dependent manner. Furthermore, mecasin significantly suppressed the LPS-induced expression of pro-inflammatory enzymes and inflammatory mediators, and also inhibited the production of NO, PGE2, COX-2, iNOS through Nrf2-mediated HO-1 expression.

Discussion and conclusion: Mecasin, a combination of natural plant extracts, showed potent cytoprotective effects on glutamate-induced neurotoxicity in the mouse hippocampal HT22 cells, presumably through Nrf2 pathway-dependent HO-1 expression. Also, the mecasin as an anti-neuroinflammatory agent was investigated in microglia activation by LPS. We have demonstrated that Mecasin suppresses pro-inflammatory mediators through Nrf2-dependent expression of anti-inflammatory HO-1 in BV-2 microglia. These results suggest that Mecasin possesses therapeutic potentials against neurodegenerative diseases with oxidative stress and neuroinflammation.

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P252 SPECIFIC LOSS AND DYSFUNCTION OF CALRETININ INTERNEURONS IN THE SOD1^{G93A} AND HUMAN ALS CORTEX: A PRIMARY MECHANISM OF ALS?

CLARK R[1], FIELDER T[1], KING A[2], DICKSON T[1]

[1]*Menzies Research Institute,* [2]*School of Medicine, University of Tasmania, Hobart, Tasmania, Australia*

Email address for correspondence: Tracey.Dickson@utas.edu.au

Keywords: pathology, interneurons, excitotoxicity

Background: In amyotrophic lateral sclerosis (ALS), increased excitability of circuitry precedes motor neuron degeneration, suggesting that ALS results from disturbances in regulation of cell excitability. However, the mechanism of presymptomatic excitability remains unknown. There is strong clinical evidence, in both cortical and spinal regions, of reduced inhibition implicating this as a potential primary mechanism. We examined the motor and somatosensory cortex of the SOD1^{G93A} and non-transgenic mice and human ALS and control tissue for expression of interneuron-specific calcium binding and neuropeptide protein markers.

Objectives: To characterise the pathological alterations to cortical interneuron subpopulations in ALS mouse models over the time course of symptom development compared to that present in ALS tissue.

Methods: Cortical tissue from presymptomatic (8 week) and end-stage (20 week) SOD1^{G93A} and ALS human cortex were serially sectioned (40μm), alongside age-matched controls, and immunohistochemically labelled with antibodies against calretinin (CR), parvalbumin (PV), somatostatin (SOM), Neuropeptide Y (NPY) and Vasoactive Intestinal protein (VIP). Morphometric analysis was also performed using Neurolucida to investigate changes in neurite length and branching patterns.

Results: In SOD1 mice we find that in late symptomatic stages, specific interneuron subsets of the motor cortex display contrasting (and potentially complimentary) pathology; the density of calretinin (CR) populations is significantly reduced by 37% in supragranular lamina (WT, $n = 4$, $55.3 \pm 6.9 mm^2$; SOD1, $n = 4$, $35.3 \pm 6.0 mm^2$), whilst neuropeptide Y (NPY) populations are increased by 40% in the infragranular lamina (WT, $n = 4$, $18.6 \pm 2.4 mm^2$; SOD1, $n = 4$, $31.0 \pm 4.2 mm^2$) ($p < 0.05$, two-way ANOVA with Bonferroni post-hoc test). Moreover, using morphometric approaches, we show that remaining CR populations have undergone early and continuing alteration to neurite labelling patterns, with progressive reductions in neurite complexity from presymptomatic- to late-symptomatic stages.

Discussion and conclusion: These findings indicate that inhibitory regulation of cortical circuitry may be impaired in a motor- and lamina-specific manner, prior to motor neuron loss, in ALS. Differential involvement of CR- and NPY-positive interneurons suggests interplay of these specific populations may drive altered regulation, as the majority of remaining cortical interneuron populations are not affected. Furthermore, analysis of human ALS post-mortem brain tissue revealed a cluster of ALS cases with reduced CR density in lamina II/III compared with controls. This may be suggestive of unique motor system vulnerabilities involving the early susceptibility of interneurons in the pathogenesis of ALS.

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P253 THE ELECTROPHYSIOLOGICAL PROPERTIES OF THE DIFFERENT MOTOR UNIT SUBTYPES ARE NOT EQUALLY AFFECTED IN ADULT SOD1-^{G93A} MICE

MANUEL M[1,2], HECKMAN CJ[2], ZYTNICKI D[1]

[1]*Univ Paris Descartes, UMR CNRS 8119, Paris, France,* [2]*Dept of Physiology, Northwestern University, Chicago, IL, USA*

Email address for correspondence: marin.manuel@neurobio.org

Keywords: excitotoxicity, spinal motoneuron, intracellular recording

Background: One of the proposed mechanisms to explain the death of motoneurons (MNs) in ALS is an excitotoxic process, ie, an excess electrical activity leading to an overload of intracellular calcium triggering apoptotic death. However, we have recently shown (1) that spinal MNs of adult mice are not intrinsically hyperexcitable in SOD1^{G93A} mice. Instead, we found that a subpopulation of MNs lost their ability to fire repetitively in response to a stationary input. Yet, at the time, we could not determine whether this hypoexcitability was restricted to a specific physiological type of motor unit.

Objectives: The goal of this work is to correlate changes in excitability with the physiological type of the recorded motor units.

Methods: We have developed an *in vivo* adult mouse preparation allowing simultaneously recording intracellularly spinal MNs and recording the force developed by their motor unit. We characterized the properties of motor units (MUs) from the Triceps Surae muscles of SOD1 mice and their non-transgenic controls (WT) at the stage preceding their denervation (P35-P65). We classified the motor units in physiological types ("Slow" - S, "Fast fatigue-Resistant" - FR and "Fast Fatigable" - FF) on the basis of their contractile properties (contraction time, twitch force, fatigability, sag in unfused tetanus).

Results: During the time frame studied, the contractile properties of the MUs are not affected by the disease, and we could use the same criteria to classify MUs as in WT mice. We found that many electrical properties of SOD1 MNs are unchanged. For example, there were no differences between SOD1 and WT MNs in term of input conductance or rheobase in each of the physiological types taken separately. However, in keeping with our earlier results, we found that a large proportion of MNs (33% vs. 9% in WT mice) lost the ability to produce a sustained firing in response to a stationary input. Interestingly, all of these MUs had a fast contraction time (mean ± SD 13 ± 3 ms, $N = 13$), and produced twitch forces ranging from

0.4 to 34 mN. Overall 4 out of the 6 MUs that we classified as FF units, and 9 out of the 18 classified as FR units had lost the ability to produce a sustained firing.

Discussion and conclusion: We were therefore able to demonstrate that FF and FR MUs (that are vulnerable in ALS), but not the S type MUs (that are resistant in ALS), become progressively hypoexcitable before they lose their connections to their muscle fibers. We are now investigating if an excitotoxic process could nevertheless arise from changes in excitatory and inhibitory inputs to MNs.

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P254 VISUALIZATION OF CSMN IN THE DISEASE MODELS OF ALS USING NOVEL UCHL1-EGFP REPORTER MICE REVEALS DETAILS OF CELLULAR VULNERABILITY

GAUTAM M[1], SEKERKOVA G[2], RAVI V[3], YASVOINA M[1], JARA J[1], MARTINA M[2], OZDINLER H[1]

[1]*Department of Neurology,* [2]*Department of Physiology, Northwestern University, Chicago, IL, USA,* [3]*Illinois Math and Science Academy, Chicago, IL, USA*

Email address for correspondence: ozdinler@northwestern.edu

Keywords: CSMN, upper motor neurons, reporter line

Background: Corticospinal motor neurons (CSMN) are unique in their ability to collect, integrate, translate and transmit cerebral cortex input towards spinal cord targets. Their location in cerebral cortex and their complex network with neighbouring cells make it extremely arduous to study CSMN specifically. Progressive degeneration of CSMN is prominent feature in many neurodegenerative diseases such as ALS. Despite their immensely important functions, lack of appropriate methods to study CSMN hinders our knowledge about the impact of their health and diseases. TDP-43 is a DNA/RNA binding protein showing pathological signatures in ALS. There are several mutations in the *TDP-43* gene involved in ALS disease (1). In addition, mutations in the *Alsin 2* (*ALS2)* gene are responsible for juvenile primary lateral sclerosis, infantile onset ascending hereditary spastic paraplegia, and are the most common cause for autosomal recessive juvenile ALS (2). Upper motor neuron signs and bulbar symptoms are often prevalent in patients with juvenile ALS. Therefore, it is important to investigate the health of CSMN in regard to these diseases.

Objective: Here, we investigate the health, stability and cellular vulnerability of CSMN in hTDP-43^{A315T} and Alsin KO mice. We crossed UCHL1-^{eGFP} reporter mice (3) with Alsin KO and hTDP-43^{A315T} mice which overexpresses human TDP-43^{A315T} gene (4), to generate hTDP-43$^{A315T\text{-}UeGFP}$ and AlsinKO-^{UeGFP} mice respectively. In the reporter disease mice, the CSMN are genetically labelled with stable eGFP expression allowing visualization and cellular analysis of CSMN through adulthood and late ages.

Results: Our ongoing studies suggest very subtle, yet important cellular changes that occur in CSMN. There is axonal degeneration of the subcerebral projection neurons, including CSMN, increased autophagy with age and changes in the localization of autophagic vesicles toward apical dendrites. Even though the neurons are not completely cleared from the motor cortex, detailed cellular visualization and analysis using immunocytochemistry coupled with electron microscopy (EM) reveal very precise aspects of cellular vulnerability and ongoing degeneration.

Discussion and conclusion: Investigation of pure upper motor neuron defects in mouse is challenging, but here we demonstrate that using UCHL1-eGFP mice as a reporter for CSMN, their health and potential pathways that contribute to their vulnerability can be studied at a cellular level with high precision.

Acknowledgements: We thank Dr. T. Siddique for providing Alsin$^{-/-}$ mice. This work was supported by the grants from Les Turner ALS Foundation and Herbert C Wenske Foundation (PHO), and NIH-R21 NS085750-01 (PHO).

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P255 IMPORTANCE OF UCHL1 FUNCTION FOR THE MOTOR NEURON CIRCUITRY AND THE HEALTH OF THE CORTICOSPINAL MOTOR NEURONS

GENC B[1], JARA J[1], ULUPINAR E[2], MANUEL M[3], COX G[4], BOHN M[1], HECKMAN CJ[3], ROOS R[5], MACKLIS JD[6], DIDONATO CJ[7], OZDINLER H[1]

[1]*Department of Neurology, Northwestern University, Chicago, IL, USA,* [2]*Osmangazi University Medical School, Eskisehir, Turkey,* [3]*Department of Physiology, Northwestern University, Chicago, IL, USA,* [4]*The Jackson Laboratories, Bar Harbor, ME, USA,* [5]*Department of Neurology, University of Chicago, Chicago, IL, USA,* [6]*Department of Stem Cell and Regenerative Biology, Harvard Medical School, Boston, MA, USA,* [7]*Department of Pediatrics, Northwestern University, Chicago, IL, USA*

Email address for correspondence: ozdinler@northwestern.edu

Keywords: corticospinal motor neurons CSMN, upper motor neurons, UCHL1

Background: Corticospinal motor neurons (CSMN) receive, integrate and relay cerebral cortex input towards spinal targets to initiate and modulate voluntary movement. CSMN degeneration is central for numerous motor neuron disorders and neurodegenerative diseases, but the cellular and molecular basis of CSMN vulnerability and progressive degeneration remains unknown. Mutations in the ubiquitin C-terminal hydrolase-L1 (*UCHL1*) gene have been detected in patients with neurodegenerative disease that affect motor function; recently three siblings displayed early neurodegeneration, including upper motor neuron dysfunction. In the absence of UCHL1 function, CSMN show profound degeneration, it is important to understand the importance of CSMN for the health of the motor neuron circuitry, and how UCHL1 function affects CSMN health and stability

Objectives: This study aims to understand the possible function of UCHL1 for the health and stability of CSMN, as well as the proper function of the motor neuron circuitry.

Methods: Using the *UCHL1*^{nm3419} mice, which lack all UCHL1 function, we have analyzed motor neuron function at multiple levels: 1) behavioural tests to evaluate overall motor function (rotarod, Digigait, and grip test); 2) *in vivo* analysis of spinal motor neuron function; 3) immunocytochemical analysis of muscle, spinal cord and cerebral cortex in combination with retrograde labelling of CSMN approaches by Fluoro-Gold and retrograde transduction via AAV2-2; 4) generated novel conditional mutant mice in which UCHL1 function is removed either from the corticospinal or the spinal motor neurons, respectively.

Results: Our results demonstrate a unique function of UCHL1 in maintaining CSMN viability and cellular integrity. *Uchl1*^{nm3419} (UCHL1 $^{-/-}$) mice, which lack all UCHL1 function, display motor neuron circuitry defects. Even though spinal motor neurons remain intact with subtle dysfunction, CSMN show early, selective, progressive and profound cell loss. CSMN degeneration is mediated via increased ER stress and becomes evident at pre-symptomatic stages by cytoarchitectural defects primarily involving the apical dendrites. The novel transgenic mice in which UCHL1 function is selectively blocked in CSMN began to reveal the impact of the CSMN health on motor neuron circuitry.

Conclusion: We report that UCHL1 is essential for motor circuitry and is especially important for CSMN health. We now characterize a novel tool especially for motor neuron diseases with prominent CSMN involvement.

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P256 DEVELOPMENT AND CHARACTERIZATION OF MUTANT HUMAN PROFILIN1 TRANSGENIC MICE AS NEW MODEL FOR ALS

YADAV S, KIAEI M

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

Email address for correspondence: syadav@uams.edu

Keywords: profilin1, actin binding protein, transgenic mouse model

Background: The mechanism of neuronal degeneration and muscle atrophy in ALS is poorly understood. So far, ALS genes identified account for approximately only 50% for familial ALS patients. Recently, five mutations in profilin1 (*PFN1)* gene (ALS18) were linked to a subpopulation of fALS patients which had none of the previously known mutated genes in fALS (1). PFN1 is an Actin-binding protein essential for regulation of filamentous F-actin formation from monomeric G-actin. Whether *PFN1* 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 average age of onset than other ALS patients, and common clinical limb onset makes a strong case for its involvement.

Objectives: To develop animal model overexpressing profilin1 with one of the mutations found in ALS patients and study whether expression of mutant profilin1 cause ALS-like phenotypes.

Methods: Standard transgenic methodology was employed and transgenic mice were monitored for general beings, behavior, weights, motor performance and survival length with standard techniques.

Results: We have successfully created three lines of transgenic mice overexpressing mutant human PFN1, High (H), Medium (M) and low (L) expressing lines. While these mice are healthy enough to breed and generate viable offspring, the H line mice develop ALS-like phenotypes; ie, hindlimb tremor, clasping, gait abnormality leading to low body profile, reduced stride length, gradual weakness and atrophy in muscle of limbs, hunched back posture (kyphosis) toward later part of the disease, and reduced life-span. Staining with the astrocyte marker, glial fibrillary acidic protein (GFAP), also indicate astrocytosis. The average survival is 177 ± 5 days for H line ($n = 9$), so far. The other two lines have subtle phenotypes that progress slowly and are still alive at 430 days, thus far.

Discussion and conclusion: We have generated three lines of transgenic mice overexpressing mutant human profilin1. Two low expressing lines have subtle phenotypes, slow progression and no change in survival length observed. The high expressing line exhibit subtle phenotype as early as weaning age and progressed slowly. They become progressively worse from 140 days, began rapid weight loss and motor weakness which resulted in premature death. To our knowledge this model with profilin1 mutation is the first to be produced and develop symptoms and signs that resembles ALS.

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P257 GENERATION OF A NOVEL MOUSE MODEL OF FUS-ALS USING BACTERIAL ARTIFICIAL CHROMOSOME (BAC) TECHNOLOGY

VIZARD T[1], KENT L[1], THOMAS M[2], 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, University of Oxford, UK, Oxford, UK,* [4]*Department of Neuropathology, John Radcliffe Hospital, Oxford, UK*

Email address for correspondence: thomas.vizard@ndcn.ox.ac.uk

Keywords: FUS, mouse, BAC

Background: Mutations in the gene encoding the RNA-binding protein FUS (Fused in sarcoma) cause a subtype of ALS characterized by cytoplasmic mislocalisation of FUS, the

extent of which correlates with disease onset and severity. A number of rodent models have been created in an attempt to recapitulate key features of FUS-ALS. The majority of these have been generated using cDNA-based expression strategies, where a FUS cDNA transgene is often expressed at levels higher than that of endogenous FUS. Expression of a wild-type FUS transgene using this approach can result in a neurodegenerative phenotype in rodents, albeit with later onset than that of an ALS-associated FUS mutant. This observation highlights whether these models truly reflect the human disease or instead demonstrate toxicity from FUS overexpression. Here we sought to generate a novel FUS mouse model relevant to human disease using the entire human FUS genomic locus together with BAC technology to express the human FUS transgene in a physiological manner.

Objectives: To create a mouse model of FUS-ALS by expressing FUS from the entire human FUS gene using BAC transgenesis.

Methods: BAC constructs containing the entire human FUS locus were generated harbouring either the human wild-type (WT) FUS sequence or the ALS-derived P525L mutation. The FUS gene including its introns, downstream of its own promoter and regulatory sequences, was cloned into a BAC. An N-terminal mCherry fluorescent tag was fused to Exon 1 of human FUS and LoxP sites were introduced flanking the FUS promoter. Founder mice were generated from pronuclear injection of fertilized oocytes with WT FUS-BAC and P525L FUS-BAC. Independent mouse lines were established from each founder.

Results: We have successively generated independent mouse lines from pronuclear injections of WT and P525L FUS-BAC constructs. Presence of the intact BAC was determined using PCR from Founder and F1 mouse genomic DNA. FUS-BAC copy number in each of the mouse lines has been determined using a qPCR assay and has shown to be present in the mouse genome from ~9 to 90 copies. Successful transgene expression has been established by Western blot and correlates with FUS-BAC copy number. Motor neurons cultured from the spinal cord of WT FUS-BAC mice reveal a predominantly nuclear localisation of human WT FUS. P525L FUS-BAC motor neurons, however, demonstrate mislocalisation of mutant FUS to the cytoplasm, consistent with the established cellular phenotype associated with this mutation.

Discussion and conclusion: Here we describe the generation of a novel FUS mouse model using BAC transgenesis. Early experimental evidence reveal that mouse lines from each of the WT and P525L FUS-BAC constructs show successful expression of the transgene.

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P258 AN INVESTIGATION OF THE ROLES OF TDP-43 AND C9ORF72 IN REGULATION OF THE NEURONAL CYTOSKELETON IN AMYOTROPHIC LATERAL SCLEROSIS

BASKARAN P, SHAW CE, GUTHRIE S

Kings College London, London, UK

Email address for correspondence: pranetha.p.baskaran@kcl.ac.uk

Keywords: TDP-43, C9ORF72, toxicity

Background: The presence of ubiquitinated misfolded protein inclusions in the cytoplasm/nucleus of neurons is the key feature of most neurodegenerative diseases. This project will directly investigate the molecular mechanisms underlying amyotrophic lateral sclerosis (ALS). Mutations in the TDP-43 and C9ORF72 genes have been shown to cause ALS. TDP-43 has DNA and RNA binding properties, and is involved in RNA splicing, transport and stability (1, 2).

Local translation of mRNA plays a key role in axonal guidance to targets, synapse formation and maintenance and neuronal survival processes which may mis-function in ALS. Genetic studies have also identified expansion repeat number as a key factor in several degenerative diseases and currently the GGGGCC (G_4C_2) intronic repeat expansion within C9ORF72 has been identified as the most common genetic cause of ALS and frontotemporal dementia (FTD) (3,4) .

Objectives: Intranuclear neuronal RNA foci have been observed in ALS and FTD tissues, suggesting that G_4C_2 RNA may be toxic. We are using rat primary cortical neurons *in vitro* and the chick embryonic system *in vivo* to model the acute effects of TDP-43 and C9ORF72 hexanucleotide repeats. We aim to test the role of TDP-43 in subcellular localisation of a key range of mRNA species, whose mis-localization can cause defective cytoskeletal dynamics, synaptogenesis/synapse maintenance and neuronal death.

Results: Our experiments show that that TDP-43 mis-localizes to the cytoplasm over time and that it directly or indirectly affects mRNA localization and axonal transport thereby affecting the cytoskeletal organization. We also demonstrate that the G4C2 repeats implicated in C9ORF72 pathogenesis form intranuclear RNA foci that initiate apoptotic cell death in chick spinal motor neurons *in vivo*.

Discussion and conclusion: The phenotypic changes observed in the motor axon projections in embryos transfected with the G_4C_2 repeats are similar to those measured in embryos transfected with TDP-43 mutations, which suggests that there may be some shared mechanism, possibly involving the sequestering of the TDP-43 protein. We propose that RNA toxicity and protein sequestration may disrupt RNA processing, along with the disruption of cytoskeletal integrity, may contribute to neurodegeneration.

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P259 TDP-43 AND THE AXONAL CYTOSKELETON

KING A, LIU Y, ATKINSON R, VICKERS J

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

Email address for correspondence: kingae@utas.edu.au

Keywords: TDP-43, axon, cytoskeleton

Background: TDP-43 is an RNA/DNA binding protein with a primary pathogenic role in ALS. Although a ubiquitously expressed protein, studies have recently shed light on how TDP-43 may have specialised functions in neurons. TDP-43, although predominantly a nuclear protein, is transported into axons and dendrites where it is involved in the transport of mRNA. Axonal and dendritic protein synthesis enables rapid alterations in local structure and directly results in adaptive responses 'on site'. TDP-43 is a known regulator of neurofilament light (NFL) mRNA thus we propose that TDP-43 plays a role in plasticity and remodelling through modulation of the cytoskeleton.

Objectives: We aim to investigate the role of TDP-43 in cytoskeletal alterations, axonal function and plasticity. In preliminary investigations, expression of TDP-43 decreased during mouse development as expression of neurofilament increased. We hypothesised that TDP-43 may be a plasticity-related protein and that increased demand for neurofilament or other cytoskeletal proteins due to neurite outgrowth or remodelling could drive increased expression of TDP-43. Therefore we investigated whether decreased expression of neurofilament protein results in alterations to TDP-43 expression, localization or phosphorylation.

Methods: We investigated TDP-43 expression in the cortex, hippocampus and spinal cord of adult (10 week) and aged (12 month) mice lacking NF-L protein. Western blotting and immunohistochemistry was performed using antibodies against phosphorylation independent TDP-43 and phosphorylated forms of TDP-43 implicated in ALS ($n = 5$ animals per group)

Results: There was a significant ($p < 0.05$) increase in TDP-43 in the spinal cord of adult mice ($49 \pm 0.08\%$) and in cortex and lumbar spinal cord of aged NFL-KO mice ($20 \pm 0.05\%$, $41\% \pm 0.09\%$ respectively). Increased TDP-43 expression was not associated with increased cytoplasmic localization of TDP-43 or with alterations in TDP-43 phosphorylation. However, altered phosphorylation of TDP-43 was associated with ageing with a significant ($p < 0.05$, $241 \pm 0.07\%$) increase in phospho-TDP-43 (pS409/410) and a significant decrease ($p < 0.05$, $22.82 \pm 0.03\%$) in phospho TDP-43 (pS403/404) in aged mice. Our current studies continue to investigate the role of altered TDP-43 (increased/decreased expression or mutation) on axon dynamics and plasticity.

Discussion and conclusion: Developmental expression of TDP-43 declines in association with the increased expression of neurofilament proteins within the axon, with the latter linked with reduced neurite plasticity and the stabilization of networks. Our study suggests that decreased expression of neurofilament can drive alterations in TDP-43 expression in vulnerable regions of the nervous system. However, in our study, increased expression of TDP-43 alone did not r esult in pathological alterations including TDP-43 phosphorylation.

Investigating the role of TDP-43 in plasticity dependent remodelling could lead to new avenues for investigating the cause of neuronal dysfunction in ALS.

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P260 SYNAPTIC ALTERATIONS IN THE TDP-43A315T MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

BLIZZARD C, HANDLEY E, CLARK R, FIELDER T, DICKSON T

University of Tasmania, Hobart, Tasmania, Australia

Email address for correspondence: Catherine.Blizzard@utas.edu.au

Keywords: TDP-43 mutation, synapse, YFP

Background: TDP-43 is the major component of the inclusions that define ALS pathologically and has been identified as a genetic cause for ALS, highlighting the importance of this protein in this disease (1). Whilst previous research has focused upon the role of TDP-43 in the nucleus under normal conditions and cytoplasmic aggregates pathologically, recent research has indicated that TDP-43 misprocessing, as a consequence of either TDP-43 mislocalisation or TDP-43 mutant gain/loss of function may have an underappreciated pathological role at the synapse

Objectives: To characterise the pre- and post-synaptic pathology occurring in the TDP-43^{A315T} mouse model of ALS with regional immunohistochemistry (IHC) and spine density analysis. Spine analysis was investigated in TDP-43^{A315T} YFP-H fluorescent mice. YFP-H mice ubiquitously express yellow fluorescent protein (YFP) in a subset of neocortical pyramidal neurons (2), making it an ideal model to study spine morphology.

Methods: Mice were perfused with 4% paraformaldehyde over a time-course of disease (postnatal day 30 to day 90). Pre- and post-synaptic pathology was investigated using IHC on 60µm coronal sections at day 90 with antibodies directed again VGlut (pre-synaptic excitatory) and GAT1 (pre-synaptic inhibitory) and PSD-95 (post-synaptic). Dendrite spines in the TDP-43^{A315T} YFP-H mice were investigated in 20µm coronal sections on the Zeiss LSM 510 Meta confocal microscope and Neurolucida software.

Results: Our investigations have identified a significant ($p < 0.05$) reduction in Glutamatergic (excitatory) and GABAergic (inhibitory) pre-synaptic vesicle transporters by day 90 (symptom onset) in the TDP-43^{A315T} mice compared to wild-type controls. Of note, these changes were specific to the motor cortex and not present in the somatosensory cortex. Additional IHC investigations revealed that PSD-95, which activity dependently co-localises with TDP-43 (3), was mislocalised to cytoplasmic granules in cortical neurons of the symptom onset TDP-43^{A315T} mice. Preliminary investigations in TDP-43^{A315T} xYFP-H mice found dendrite spine alterations during symptom onset, suggesting that early post-synaptic disturbances may be occurring in this model. We are currently using this novel mouse model to determine the precise time-course of spine alterations using multi photon *in vivo* live imaging.

Conclusion: Synaptic dysfunction is an early pathogenic event in ALS. Our investigations highlight a potential pathogenic role for TDP-43 at the synapse. Understanding the role that TDP-43 plays in this synaptic pathogenesis represents a critical first step towards revealing a new therapeutic window for intervention - targeted at synaptic function - to improve outcomes for people suffering from ALS.

Acknowledgements: This work was supported by the MNDRIA, Alzheimer's Australia and Brain Foundation Australia.

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P261 TDP-43-MEDIATED HDAC6 EXPRESSION PROMOTES THE FORMATION OF LARGER PRE-SYNAPTIC DENSITIES, FACILITATING NEUROTRANSMITTER RELEASE

MISKIEWICZ K[1,2], JOSE L[1,2], YESHAW W[1,2], VALADAS J[1,2], SWERTS J[1,2], MUNCK S[2,3], FEIGUIN F[4], DERMAUT B[5,6], VERSTREKEN P[1,2]

[1]*VIB, Center for the Biology of Disease, Leuven, Belgium,* [2]*KULeuven, Center for Human Genetics, Leuven, Belgium,* [3]*VIB Bio Imaging Core, Leuven, Belgium,* [4]*International Center for Genetic Engineering and Biotechnology, Trieste, Italy,* [5]*Institut Pasteur de Lille, Université de Lille Nord de France, Lille, France,* [6]*Center for Medical Genetics, Ghent University Hospital, Gent, Belgium*

Email address for correspondence: Katarzyna.Miskiewicz@cme.vib-kuleuven.be

Keywords: TDP-43, active zone, neurotransmission

Background: TAR DNA-binding protein TDP-43 acts in different neurodegenerative diseases and mutations in TDP-43 are causative for amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration. Furthermore the early phases of ALS and other neurodegenerative disorders are characterized by hyper-excitability but the mechanisms resulting in synaptic defects remain enigmatic.

Objectives: The aim of this work is to identify early synaptic defects caused by deregulation of TDP-43/TBPH function and to characterize the mechanisms of synaptic dysfunction.

Methods: We used the neuromuscular junction of *Drosophila* 3rd instar larvae expressing pathogenic TDP-43 mutants and *tbph*/*tdp-43* null mutants. We applied super-resolution microscopy and TEM to identify structural changes at synapses as well as synaptopHluorin imaging and electrophysiology to characterize pre-synaptic function.

Results: The expression of pathogenic mutants TDP-43 results in increased synaptic vesicle fusion and a larger readily releasable pool. These defects correlate with enlargement of synaptic vesicle-tethering sites. In contrast *tbph* loss-of-function mutations cause opposite defects, suggesting that pathogenic TDP-43 expression causes synaptic defects by a gain-of-function mechanism.

The expression of pathogenic TDP-43 mimics phenotypes that were previously linked to the reduction in the acetylation status of the protein bruchpilot (BRP), which is a component of the vesicle tethering site at synapses (1). Using biochemical assays we show that flies expressing pathogenic TDP-43 show much lower BRP acetylation levels *in vivo*. TDP-43 is known to bind HDAC6 mRNA and regulates its expression such that *tbph* loss-of-function results in low levels of HDAC6, while over-expression of pathogenic and wild type TDP43 causes up-regulation of HDAC6 mRNA. Using *in vivo* and *in vitro* assays we show that HDAC6 is necessary and sufficient to de-acetylate BRP, providing an explanation as to why BRP acetylation is reduced in animals expressing TDP-43. Furthermore, our data indicate that the synaptic defects upon expression of TDP-43 pathogenic mutants correlate with HDAC6 up-regulation, as over expression of HDAC6 also causes larger tethering sites and more synaptic transmission while HDAC6 knock out causes opposite phenotypes, similar to *tbph* loss-of-function. Finally, genetically correcting the BRP acetylation defects in TDP-43 mutant expressing animals using partial loss of *hdac6* or over-expression of ELP3, a BRP acetyltransferase also associated with ALS, rescues the synaptic and adult motor defects.

Discussion and conclusion: We show that de-regulation of TDP-43/TBPH cause defects in neuronal transmission by controlling HDAC6 expression. We identify HDAC6 as a de-acetylase of the active zone protein BRP. Furthermore, our work using fruit flies points to the convergence of two ALS-relevant players, TDP-43 and ELP3, suggesting that HDAC6-dependent de-acetylation and ELP3-dependent acetylation of active zone material is a mechanism by which synaptic vesicle tethering and fusion are regulated.

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P262 STRUCTURE-TOXICITY STUDY OF FUS USING DROSOPHILA AS A MODEL SYSTEM

WILMANS N[1,2], BOGAERT E[1,2], SCHEVENEELS W[1,2], VAN DAMME P[1,3], ROBBERECHT W[1,3], CALLAERTS P[4], VAN DEN BOSCH L[1,2]

[1]*Vesalius Research Center, Leuven, Belgium,* [2]*Experimental Neurology (Department of Neurosciences) and Leuven Institute for Neuroscience and Disease (LIND), University of Leuven (KULeuven), Leuven, Belgium,* [3]*Neurology, University Hospitals Leuven, Leuven, Belgium,* [4]*Laboratory of Behavioural and Developmental genetics VIB, Leuven, Belgium*

Email address for correspondence: elke.bogaert@med.kuleuven.be

Keywords: FUS, Drosophila

Background: A subgroup within the ALS/FTD disease spectrum, the FUSopathy, is characterized by FUS inclusions in neurons and glial cells. The observation that mutations in FUS cause ALS emphasizes the involvement of FUS in the disease pathogenesis.

Objectives: We aimed to identify toxicity domains in FUS protein.

Methods: In order to examine the pathogenic role of FUS, we generated four different transgenic fly lines, allowing expression of wild type human FUS (WT hFUS) and three disease-associated mutant human FUS proteins ($hFUS^{R521G}$, $hFUS^{R521H}$ and $hFUS^{P525L}$). Selective expression of hFUS transgenes in adult motor neurons via the UAS-GAL4 expression system resulted in a progressive decline of motor performance ultimately leading to early death of the flies.

Results: Overexpression of human FUS in flies leads to severely reduced life span and motor performance defects. However, no differences were observed between wild type and mutant FUS overexpressing flies. To address the toxicity arising from human wild type FUS, we deleted all functional domains from the wild type protein and addressed toxicity in both animal models. We identified important domains present in the N terminal and C terminal part of the full-length pro-

tein. In a next set of experiments, we identified the minimal region required for FUS toxicity by evaluating the toxicity arising from overexpression of these domains.

Discussion and conclusion: We used *Drosophila* as a model system to gain insight into the pathogenic mechanism of FUS induced motor performance defects. Functional regions in the N terminal and C terminal part of the protein are pivotal for toxicity in fly motor neurons.

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P263 IDENTIFYING GENETIC SUPPRESSORS OF TDP-43 TOXICITY USING DROSOPHILA

SREEDHARAN J[1,2], NEUKOMM L[1], COLEMAN M[2], BROWN R[1], FREEMAN M[1]

[1]*UMass Medical School, Massachusetts, USA,* [2]*Babraham Institute, Cambridge, UK*

Email address for correspondence: jemeen@hotmail.com

Keywords: TDP-43, axon, Drosophila

Background: Pathological and genetic studies suggest that TDP-43 is a final common mediator in sporadic and familial ALS. Understanding TDP-43 pathobiology is a central aim of ALS research but is complicated by the diverse roles of this protein in nuclear, cytoplasmic and axonal compartments of neurons. Determining realistic therapeutic targets will require *in vivo* approaches that model the earliest changes seen in ALS, such as synapse and axon degeneration. *Drosophila* offers unrivalled genetic tools to conduct these studies and, importantly, TDP-43 is structurally and functionally conserved in flies. However, while existing fly models recapitulate TDP-43 toxicity there has been limited progress in identifying suppressors of toxicity. This may be because these models focus on eye and larval neuronal phenotypes. A better approach would be to study adult motor neurons, the principle cell type afflicted in ALS.

Objectives: i) Develop a strategy to examine adult *Drosophila* motor neurons *in vivo*; ii) Use this approach to model progressive TDP-43-mediated motor neuron degeneration; iii) Perform a forward screen for novel genetic suppressors of TDP-43 toxicity.

Methods and results: We used mosaic analysis with a repressible cell marker (MARCM) to visualize motor neurons with single cell resolution in the adult fly using the glutamatergic OK371-Gal4 driver(1). MARCM clones were induced using flippase under the control of a proneural gene promoter (2). Confocal microscopy was used to resolve axons, neuromuscular junctions (NMJs) and active zones without the need for complex dissection or immunostaining. Overexpression of mutant TDP-43 caused progressive 'dying back' neurodegeneration, which preferentially affected motor rather than sensory neurons and could be monitored over two months (the lifetime of the fly). We then screened for novel suppressors of this phenotype using ethyl methanesulphonate (EMS) mutagenesis. After examining over 3000 EMS lines three recessive suppressors of TDP-43 toxicity were isolated and identified through duplication mapping and whole genome resequencing. These loss-of-function hits implicate chromatin, RNA and microtubule biology and are undergoing further validation in mammalian neurons expressing mutant TDP-43.

Discussion and conclusion: Our mosaic approach to studying neurodegeneration permits, for the first time, a simple method of studying individual motor neurons, axons and NMJs *in vivo*. MARCM permits the long-term study in adult flies of toxic proteins such as TDP-43, which are otherwise lethal at prepupal stages if expressed ubiquitously. Similarly, MARCM makes it possible to isolate recessive suppressors of TDP-43 toxicity, which would be missed using other screening methods. Only 20% of the fly genome was evaluated in this study and many other suppressors of TDP-43 toxicity await identification.

Acknowledgements: Medical Research Council, Motor Neurone Disease Association, Howard Hughes Medical Institute, Max Rosenfeld Fund, UMMS

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THEME 11 THERAPEUTIC STRATEGIES

P264 GENE THERAPY FOR SPORADIC ALS USING AN INTRAVENOUS INJECTION OF AAV VECTOR

YAMASHITA T[1], CHAI HL[1], TERAMOTO S[1], MURAMATSU S-I[2] , KWAK S[1]

[1]*University of Tokyo, Tokyo, Japan,* [2]*Jichi Medical University, Tochigi, Japan*

Email address for correspondence: kwak-tky@umin.ac.jp

Keywords: adeno-associated virus (AAV), AMPA receptor, ADAR2

Background: Amyotrophic lateral sclerosis (ALS) affects the middle-aged and elderly and is characterized by progressive muscular weakness resulting from degeneration of motor neurons in the spinal cord and the brain stem. There is no known cure and the patients die from respiratory muscle failure within a few years after onset. Downregulation of the RNA editing enzyme ADAR2 is involved in the death of motor neurons of sporadic ALS patients, which accounts for the great majority of cases of the disease. Therefore, normalization of ADAR2 activity in motor neurons is a likely therapeutic strategy for ALS.

Objectives: We developed an adeno-associated virus serotype 9 (AAV9) vector that would enable gene delivery only to the neurons of the brain and spinal cord via intravenous injection. We investigated whether delivery of the ADAR2 gene to motor neurons using the AAV9 vector would prevent the progression of symptoms of the disease and the degeneration of motor neurons in mechanistic model mice of sporadic ALS (conditional ADAR2 knockout mice or AR2 mice).

Results: Expression of the ADAR2 gene in motor neurons stopped the process leading to cell death and symptoms due to effective prevention of cell death without any apparent side-effects, even when administered after the emergence of symptoms,. Through the use of the AAV9 vector to trigger gene expression only in neurons, we could overcome the difficulty to introduce genetic material into the brain and spinal cord by intravenous injection, and demonstrate that one intravenous injection alone was sufficient to bring about long-lasting expression of an effective quantity of the ADAR2 gene.

Discussion and conclusion: While this result was achieved with a model mouse, it is thought that a similar molecular mechanism underlies sporadic ALS in human patients, and as the human ADAR2 gene had a therapeutic effect in the model mouse, it is anticipated that a similar form of gene therapy will be effective in treating human ALS as well. Further, the AAV9 vector is known to be safe, and after confirming the safety of the improved AAV9 vector and determining optimal dosage, it is hoped that this research will open a new route to the treatment of ALS. Currently gene therapy has a strong image as a replacement therapy for rare genetic disorders, but this research is unique in that it shows that gene therapy is possible even in sporadic cases if the molecular pathology of the disease is understood.

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P265 ADENOVIRAL TARGETING OF THE MOTOR END PLATE REGION FOR INCREASED TRANSDUCTION OF MOTOR NEURONS AND SKELETAL MYOFIBRES

TOSOLINI A, MOHAN R, MORRIS R

Translational Neuroscience Facility, School of Medical Sciences, UNSW Australia, Sydney, NSW, Australia

Email address for correspondence: andrew.tosolini@unsw.edu.au

Keywords: gene therapy, motor neurons, adeno-associated virus (AAV)

Background: Gene therapy is an exciting technique that has the capability to introduce novel therapeutic genes that can either maintain or re-establish functional connectivity in a deficient system. Various approaches previously used for the treatment of rodent models of ALS have included the delivery of trophic factors (eg, insulin-like growth factor, etc.) or have used siRNA to knockdown mutant defective genes (eg, SOD1, etc.). In a gene therapy scenario, intramuscular injections and the subsequent retrograde transport of a viral vector is a minimally invasive way to transduce both the innervating motor neuron and the skeletal muscle. We have previously shown that targeting the entire length of muscles' motor end plate (MEP) region significantly increase the uptake of a retrograde tracer into corresponding motor neurons (1,2,3). However, using the MEPs as a target to deliver viruses into the spinal cord motor neurons has not yet been explored.

Objectives: The aim of this study was to determine if targeting the entire MEP region of a muscle with adenovirus would, as is the case with retrograde tracers, significantly increase expression of the transgene within spinal cord motor neurons in the wild-type mouse.

Methods: Recombinant adenovirus serotype 5 driven by the CMV promoter and encoding the reporter tag-GFP (Ad-GFP) was obtained through the UPenn vector core. Using our recently published MEP map as a guide (2), Ad-GFP (5.3×10^{12} pfu/ml) was injected along the entire MEP region along various muscles. Mice were subsequently intra-cardially perfused and the spinal cord, and injected skeletal muscles were dissected out, sectioned and the tissue were analysed under epifluorescence.

Results: This analysis showed that targeting the MEPs with Ad-GFP produced significant expression of GFP within spinal cord motor neurons and the targeted skeletal muscles. Moreover, GFP expression was also present within ventral roots, dorsal roots and dorsal root ganglia.

Discussion and conclusion: This study suggests that targeting muscles' MEP regions with an adenovirus is an effective and minimally invasive way to retrogradely deliver therapeutic

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genes into spinal cord motor neurons and skeletal myofibres. These data have implications for gene therapies aiming to maintain synaptic health between skeletal muscle fibres and the innervating spinal cord motor neurons.

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P266 SPECIFIC GENE DELIVERY TO CORTICOSPINAL MOTOR NEURONS BY AAV

JARA J[1], STANFORD M[1], ZHU Y[2], BOHN M[1], DEVRIES S[2], OZDINLER H[1]

[1]*Department of Neurology,* [2]*Department of Opthalmology, Northwestern University, Chicago, IL, USA*

Email address for correspondence: ozdinler@northwestern.edu

Keywords: corticospinal motor neurons, adeno-associated virus (AAV), motor neuron circuitry

Background: Corticospinal motor neurons (CSMN) are limited in numbers and are embedded among hundreds of different neuron types in the heterogeneous structure of the cerebral cortex. They are important for the initiation and modulation of voluntary movement as they receive, integrate, translate and transmit the cerebral cortex input towards spinal cord targets. CSMN degeneration is a hallmark for many neurodegenerative diseases and their genetic modulation is required for cellular therapies. The application of adeno-associated virus (AAV) in the central nervous system (CNS) has multiple advantages (1). AAV-IGF increased the lifespan of the well-characterized hSOD1^{G93A} ALS mouse model (2) and since then multiple therapeutic and translational studies have been proposed for motor neuron diseases. AAV serotypes are extremely malleable and combinations of promoters and capsid engineering have improved the transduction efficiency towards specific cell types. In ALS, targeting vulnerable neuron populations without affecting other neuron types within the cerebral cortex represents a major obstacle to establish new therapeutic strategies. We have recently reported specific transduction of CSMN after injection into the corticospinal tract (3). In this report, CSMN degeneration is observed associated with a distinct pattern of vacuolization that has not been reported before in the hSOD1^{G93A} ALS mouse model.

Objectives: To develop approaches that allow selective and specific gene delivery to CSMN using AAV.

Methods: We used seven AAV serotypes (AAV2-1, AAV2-2, AAV2-5, AAV2-6, AAV2-7, AAV2-8, and AAV2-9) that harbour the eGFP gene under control of the CMV promoter. AAVs were injected directly into the motor cortex in conjunction with retrograde labelling with red fluorescent microspheres to marks CSMN in the motor cortex and to investigate specific tropism for CSMN. In addition to co-labelling with red fluorescent microspheres, Ctip2 co-localization was used to confirm CSMN transduction.

Results: All AAVs tested showed varied tropism for neurons and glial cells including astrocytes. Our results clearly demonstrate the superiority of AAV2-2 for specific CSMN transduction, which is highly improved upon utilization of CBA promoter. Our results also indicated that diseased CSMN could be transduced effectively.

Discussion and conclusion: Our results suggest that the choice of the promoter is critically important to enhance selectivity on gene expression in CSMN. Identification of AAV serotypes that transduce only a select set of neuron populations, even upon direct cortical injection is critically important to develop effective and long-term gene therapy approaches in the cerebral cortex.

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P267 TARGETED NON-VIRAL GENE DELIVERY TO MOTOR NEURONS

SMITH KS[1], HOFFMAN L[3], RUSH RA[1], VOELCKER NH[2], ROGERS M-L[1]

[1]*Flinders University, Department of Human Physiology, Centre for Neuroscience, Adelaide, South Australia, Australia,* [2]*University of South Australia, Adelaide, South Australia, Australia,* [3]*South Dakota State University, Department of Chemistry and Biochemistry, Brookings, South Dakota, USA*

Email address for correspondence: kevin.smith@flinders.edu.au

Keywords: gene therapy, targeted, p75NTR

Background: Targeted gene therapy for motor neuron disease (MND)/amyotrophic lateral sclerosis (ALS) using non-viral nanoconstructs has huge potential for treatment, but so far, has underachieved. Our group has been developing non-viral gene delivery nanocarriers called 'immunogenes'. An immunogene is comprised of an internalizing antibody to a cell surface receptor that is conjugated to a polycationic carrier, which can bind and condense DNA/ RNA (1). Extensive research has revealed that the common neurotrophin receptor p75 (p75NTR) is naturally expressed in embryonic and neonatal motor neurons, as well as in adult motor neurons that are damaged or diseased, including MND/ALS. p75NTR is retrogradely trafficked in motor neuron signalling endosomes, thus we hypothesize this receptor is an ideal target for therapeutic gene delivery. Our current non-viral gene delivery agent consists of an antibody to p75NTR (MLR2) conjugated to a PEGylated polyethylenimine (PEI-PEG12) creating an immunoporter, which upon binding to a plasmid forms the immunogene. We aimed to characterise the ability of this carrier to transfect motor neurons *in vitro* and *in vivo*.

Methods: MLR2-PEI-PEG12 (immunoporter) was constructed and tested for its ability to bind/condense plasmid DNA (pVIVO2 that can express GFP) to form the immunogene and neutralise charge using a gel-retardation assay. Embryonic motor neurons were isolated from C57BL/6J (B6) mice and GFP expression was checked after application of MLR2-PEI-PEG12-pVIVO2. Finally, neonatal B6 mice were

injected intraperitoneally with MLR2-PEI-PEG12-pVIVO2 and then spinal motor neurons positive for ChAT and p75NTR were checked for GFP expression.

Results: MLR2-PEI-PEG12 was able to bind and condense pVIVO2 DNA and become charge neutral. MLR2-PEI-PEG12 successfully delivered pVIVO2 plasmid DNA specifically to primary motor neuron cultures isolated from neonatal mice and GFP was expressed. Approximately 25.4% of lumbar motor neurons in neonatal B6 mice (n = 5) identified by ChAT also expressed GFP after intraperitoneal injections of MLR2-PEI-PEG12 (150μg) carrying pVIVO2 (116μg). The GFP expressing motor neurons were also identified as expressing p75NTR.

Discussion and conclusion: We have shown that the immunogene comprising MLR2-PEI-PEG12-pVIVO2 has the ability to specifically transfect p75NTR expressing motor neurons. Further work will be needed to apply this technique to mice with MND and eventually as targeted therapy for MND/ALS.

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P268 LIPOSOME-ENCAPSULATED H-FERRITIN IMPROVES SURVIVAL IN AN SOD1 MUTANT MOUSE MODEL OF ALS

SNYDER A[1], MADHANKUMAR A[1], NEELY E[1], RIZK E[1], HESS O[2], SIMMONS Z[3], CONNOR J[1]

[1]*Department of Neurosurgery, Penn State University, Hershey, PA, USA* [2]*Mechanicsburg Area High School, Mechanicsburg, PA, USA,* [3]*Department of Neurology, Penn State University, Hershey, PA, USA*

Email address for correspondence: asnyder9@hmc.psu.edu

Keywords: iron, lipopolysaccharide, infusion

Background: The misregulation of iron and subsequent oxidative stress are consistent features shared between humans with ALS and animal models of the disease. The iron sequestration protein H-ferritin has ferroxidase activity and limits the toxic potential of iron, making it an attractive therapy to pursue in ALS. One of the disadvantages of most systemically-delivered treatments for neurological diseases is that they exert their biological effects not only at their target sites but also at peripheral tissue and cells. This often results in dilution of the agent below therapeutic levels to the target tissue; a way to reduce the amount of agent administered and thus to potentially reduce toxicity is to utilize liposomal drug carriers.

Objective: To determine if infusion of liposome-encapsulated iron-poor H-ferritin has neurorescue properties in a murine model of ALS.

Methods: At 90 days of age, mice with the $SOD1^{G93A}$ mutation underwent surgery to permit continuous infusion into the lateral ventricle. There were a total of three groups: animals that received infusion of liposome-encapsulated H-ferritin that was targeted to microglia by the presence of lipopolysaccharide (LPS) on the surface of the liposome (n = 6), animals that received infusion of non-targeted liposome-encapsulated H-ferritin (n = 10), and a No Surgery (control) group (n = 20). Disease onset was assessed by performance on the rotarod apparatus, and endpoint was determined by the inability of the animal to right itself.

Results: Treatment with H-ferritin encapsulated by non-targeted liposomes resulted in a median lifespan of 136.5 days, as compared to 128.5 and 126 days for the LPS-targeted liposome and No Surgery groups, respectively. Histological examination of lumbar spinal cord sections indicated less extensive microglial activation at end-stage in the non-targeted liposome group as compared to the LPS-directed liposome treated group. Furthermore, the motor neurons that remained at end-state in the non-targeted liposomal group had thick, extensively branched projections, which were features not seen in the LPS-targeted group.

Discussion and conclusion: We propose that the limited benefit of LPS-directed delivery of H-ferritin is due to over-stimulation of microglia by accessing them through the TLR-4 receptor. Therefore, a plausible explanation as to why the non-directed liposomes are effective is that microglia are not further activated by our therapy, yet as the main phagocytic cell type in the CNS, they readily uptake the liposomes. Our intervention in the animal model is of particular relevance to the clinical population because our intervention occurs at a stage of the disease at which individuals with ALS would begin to notice symptoms and seek treatment. Therefore, liposomal delivery of H-ferritin may be of greater clinical benefit than those compounds tested while animals are pre-symptomatic.

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P269 OPTIMAL CONDITIONS FOR TRANSPLANTATION OF MESENCHYMAL STEM CELLS IN THE ALS MOUSE MODEL

EBIKI M[1], NAKAMURA K[2], WATANABE Y[1], KAZUKI Y[3], YOSHIDA-YAMAKAWA M[1], HOSOKAWA H[1], OSHIMURA M[3], NAKASHIMA K[1]

[1]*Division of Neurology, Department of Brain and Neurosciences, Faculty of Medicine, Tottori University, Nishi-cho, Yonago, Japan,* [2]*Division of Laboratory Animal Science, Research Center for Bioscience and Technology, Tottori University, Nishi-cho, Yonago, Japan,* [3]*Department of Biomedical Science, Institute of Regenerative Medicine and Biofunction, Graduate School of Medical Science, and Chromosome Engineering Research Center Tottori University, Nishi-cho, Yonago, Japan*

Email address for correspondence: e.mitsutaka@gmail.com

Keywords: mesenchymal stem cells (MSC), neurotrophic factor, transplantation

Background: Stem cell therapy is a promising therapeutic approach for the treatment of amyotrophic lateral sclerosis (ALS). Mesenchymal stem cells (MSCs) are one of the best cell sources in such an application. We previously established an MSC clone (MSC3-31) that simultaneously overexpressed glial cell-derived neurotrophic factor, hepatocyte growth factor and insulin-like growth factor-1. This cell line provides the opportunity for stable transplantation and thorough analysis.

Objectives: The aim of this study was to optimize transplantation conditions, focusing in particular on the transplantation route as well as mouse recipient age. We compared the efficacies of transplantation using the fourth cerebral ventricle (CV) vs. intravenous (IV) injection, using mice that were 60 or 100 days old.

Methods: (1) High copy SOD^{G93A} transgenic (ALS) mice were given an oral immunosuppressive agent from one week before the transplantation until death. (2) MSC3-31 cells were transplanted into the ALS mice (60 or 100 days of age) via the CV or via IV (through a surgically exposed jugular vein). Phosphate buffered saline was given to the ALS mice (60 or 100 days) via CV or IV as control groups. (3) Clinical evaluations (body weight, hind limb extension reflex score, etc.) were performed to assess treated and control groups. (4) Immunohistochemical observations were performed on the spinal cords of both groups.

Results: In the groups transplanted via CV, there was an encouraging trend resulting in delayed death in the treated mice compared to the controls in mice transplanted at 60 days. Further, in transplants of MSC3-31 cells via CV at 100 days, ALS model mice showed a longer life span than did the control group (treated group vs. control group: 147.7 ± 2.5 days vs 140.8 ± 1.3, $p < 0.005$). For the IV groups, significant differences were not found in the 100-day-transplanted group regarding onset time or life span. Although the 60-day-treated group showed a tendency for a delayed onset time, it was not significantly different compared to the controls.

Discussion and conclusion: Encouraging trends were observed following transplantation via CV as well as IV at 60 days. Nevertheless, we conclude that optimal transplantation parameters are via CV transplantation at 100 days of age in ALS mice.

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P270 A DOSE ESCALATION SAFETY TRIAL ON INTRATHECAL DELIVERY OF AUTOLOGOUS ADIPOSE-DERIVED MESENCHYMAL STROMAL CELLS IN AMYOTROPHIC LATERAL SCLEROSIS

STAFF N, SORENSON E, BUTLER G, PADLEY D, WEIS D, GASTINEAU D, DIETZ A, WINDEBANK A

Mayo Clinic, Rochester, MN, USA

Email address for correspondence: staff.nathan@mayo.edu

Keywords: mesenchymal stromal cells, stem cells, clinical trials

Background: Mesenchymal stromal cells (MSCs) hold promise as a treatment for neurodegenerative diseases such as ALS due to their known paracrine effects on the CNS and immune system.

Objectives: We report interim results from our dose-escalation safety trial using intrathecal autologous adipose-derived MSCs (clinicaltrials.gov #NCT01609283).

Methods: Fifteen patients with ALS symptoms for 1-2 years were treated with 1-2 doses of 10, 50 or 100×10^6 MSCs via lumbar puncture. Patients were monitored for adverse events via symptom diary, clinical visits, blood, ALSFRS-R, CSF and MRI.

Results: Intrathecal MSC treatment was well tolerated, with reported mild adverse events unlikely related to treatment. At the 50×10^6 dose, most patients developed mild CSF pleocytosis (< 18 cells/uL), and one patient exhibited asymptomatic MRI lumbar nerve root thickening. No patients developed worsening weakness (more than expected with ALS), paresthesias/pain, or bowel/bladder dysfunction on follow-up (median 129 days, range 27-402 days).

Discussion and conclusion: Intrathecal autologous adipose-derived MSCs appear safe in ALS patients at the doses tested, and further clinical trials to assess efficacy should be considered.

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P271 SLOWING DISEASE PROGRESSION IN THE SOD1 MOUSE MODEL OF ALS BY BLOCKING NEUREGULIN

SONG F[1], LIU J[1], RAVITS J[2], SIMPSON E[3], LOEB J[1]

[1]*University of Illinois at Chicago, Chicago, IL 60612, USA,* [2]*ALS and Neuromuscular Translational Research University of California, San Diego, USA,* [3]*Department of Psychiatry, Columbia University, New York, USA*

Email address for correspondence: feisong@uic.edu

Keywords: neuregulin1 antagonist, therapeutic target, disease progression

Background: Neuregulin1 (NRG1) is a gliotrophic factor that regulates glial development and survival, synaptogenesis, axoglial interactions, and microglial activation. We recently found that NRG1 receptors are activated on microglia in the ventral horn of both ALS patients and in ALS-SOD1 mice (1). NRG1 signalling is also activated on microglia in the corticospinal tracts in ALS patients with predominant upper motor neuron signs (2), suggesting a common pathological mechanism (1, 2). We have developed a targeted NRG1 antagonist called HBD-S-H4 that when given intrathecally, reduced microglia activation in a rat chronic spinal cord pain model. Therefore, here we hypothesize that blocking NRG1 with HBD-S-H4 could be a new potential therapeutic treatment to slow microglial activation and disease progression in patients with ALS.

Objectives: To determine whether blocking NRG1 in the central nervous system (CNS) slows disease progression and prolongs survival in the ALS-SOD1 mouse model.

Methods: To determine if blocking NRG1 signalling would provide therapeutic benefit in the ALS-SOD1 mouse model, we used two different methods to deliver HBD-S-H4 to the CNS. In one approach we generated triple transgenic (Tg)

mice to express HBD-S-H4 in the CNS of SOD1 mice (GFAP-tTA:tetO-HBD-S-H4:SOD1^{G93A}). In an alternate approach, we injected HBD-S-H4 weekly through an implanted intracerebroventricular (icv) cannula for 9 or more weeks. Body weight, disease onset and progression, animal survival as well as pathological changes were measured in the triple Tg mice, HBD-S-H4 treated SOD1 mice and compared with their respective control groups.

Results: Our data shows that the expression of HBD-S-H4 in the CNS delays disease onset and prolongs survival in GFAP-tTA:tetO-HBD-S-H4:SOD1 mice compared with GFAP-tTA:SOD1 as well as SOD1 mice. Consistent with this therapeutic effect of transgenic expression of HBD-S-4, we found that high levels of HBD-S-H4 expression correlate with longer survival. Weekly icv treatment of recombinant HBD-S-H4 for 9 weeks had no toxic effects and was found to delay disease onset and prolong survival in the SOD1 mice. Measurements of the cellular pathology in GFAP-tTA:tetO-HBD-S-H4:SOD1 Tg mice and HBD-S-H4 icv-treated SOD1 mice are currently underway.

Discussion and conclusion: We have identified a common therapeutic target of NRG1 receptor activation on activated microglia in both ALS patients and the ALS-SOD1 mouse model. We are currently testing whether our NRG1 antagonist functions by blocking the communication between neuron and glia in the SOD1 mouse model and whether this would be a potential therapeutic treatment for patients with ALS.

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P272 ADMINISTRATION OF ANTIBODIES FOR MISFOLDED SOD1 PROLONG SURVIVAL IN SOD1 MOUSE MODELS

MCCAMPBELL A, QIAN F, EHRENFELS C, LUO Y, SUN L, ZHANG M, WEINREB P, AMBROSE C

Biogen Idec, Cambridge, MA, USA

Email address for correspondence: alexander.mccampbell@biogenidec.com

Keywords: SOD1, misfolded, antibodies

Background: Since the identification of mutations in the superoxide dismutase 1 (SOD1) gene as a cause of amyotrophic lateral sclerosis (ALS), substantial efforts have been made to understand how mutations in SOD1 trigger motor neuron degeneration. Recent work has focused on the connection between toxicity and the propensity of mutant SOD1 protein to misfold. Mice expressing mutant SOD1 recapitulate many of the pathological and clinical features of ALS. Several reports have shown that targeting SOD1 by active or passive immunization can prolong survival.

Objective: We sought to compare the performance of antibodies with varying affinity for forms of SOD1 across two different mouse models. We used SOD1^{G93A} high copy mice and SOD1^{G37R} mice.

Methods: Congenic C57Bl6 mice expressing either SOD1^{G93A} or SOD1^{G37R} were obtained from Jackson Labs. Mice expressing G93A were dosed once a week starting at 50 days old at 30 mg/kg. Mice expressing G37R were aged to 6 months prior to dosing. Animals were regularly tested for motor performance by a rotarod analysis. Body weights and clinical scores were routinely assessed. Antibodies tested in these studies include B8H10, 3H1, and MB591-37.

Results: Disease onset as assessed by time to lose 10% of peak body weight was significantly delayed in both models. Disease onset as assessed by clinical observation was delayed in the G93A model, but not the G37R model. In contrast, rotarod performance was not altered in the G93A model, but was significantly improved in the G37R mice. Antibodies improved survival in both mouse models. The absolute change was much greater in the G37R mice; however, the relative change as calculated by a percent increase in life compared to control IgG dosed mice, was very similar for both models.

Discussion and conclusion: These data are consistent with the hypothesis that misfolded SOD1 represents a toxic form of SOD1. Consistent with prior reports, administration of antibodies directed against misfolded SOD1 confers increased survival in mutant SOD1 expressing mice. While some differential effects were seen across the models, in general the performance was very comparable. The G37R mice showed greater sensitivity in the motor performance assays, but this may reflect the slower disease course as opposed to a fundamental difference in disease pathobiology.

In conclusion, treatment of SOD1^{G93A} mice with antibodies specific for misfolded SOD1 improves survival, but additional work to understand the mechanism of action is needed.

Acknowledgements: Antibodies were licensed from AviTix Inc. and Amorfix Life Sciences and obtained through an MTA with MassBiologics. All authors were full-time employees of Biogen Idec.

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P273 CHARACTERIZATION OF ANTIBODIES TO MISFOLDED SOD1

AMBROSE C, QIAN F, EHRENFELS C, LUO Y, SUN L, DUPAGE A, YANG W, TAN X, ANTOGNETTI G, DATTA A, CAMERON T, ZHANG M, AULUCK P, FARRINGTON G, MCCAMPBELL A, WEINREB P

Biogen Idec, Inc., Cambridge, MA, USA

Email address for correspondence: christine.ambrose@biogenidec.com

Keywords: antibodies, misfolded, SOD1

Background: Mutations in the Cu/Zn superoxide dismutase (SOD1) gene cause about 20% of familial or 1–2% of all ALS cases. The mutations are thought to cause a gain of function, making SOD1 more prone to aggregation, and ultimately leading to motor neuron cell death. It has been proposed that misfolded SOD1 also plays a role in sporadic ALS and that misfolded wild-type SOD1 can propagate from cell to cell in a prion-like manner. In order to target extracellular misfolded SOD1, several groups have generated antibodies to misfolded SOD1 and used them to treat mutant SOD1 transgenic mice.

Objective: To characterize a large number of antibodies generated to different misfolded or mutant SOD1 antigens, in order to select the best candidates for *in vivo* studies and to use them to develop an assay to measure misfolded SOD1.

Methods: The apparent affinities of the antibodies for guanidine-denatured, oxidized or native SOD1 were determined. Mutant SOD1 recognition was assessed by immunoprecipitation from cell lysates containing various SOD1 mutants. Epitope mapping was done using deletion or point mutants of SOD1 and with a peptide array. An ELISA to specifically quantify misfolded SOD1 using one of the misfolded Abs was developed in addition to a native SOD1 ELISA. The ELISA was applied to soluble spinal cord extracts and western blots were used to examine insoluble SOD1 prepared from longitudinal samples from 3 strains of SOD1 transgenic mice. Immunohistochemistry on G93A transgenic or control spinal cord tissue was carried out using 3 of the Abs.

Results: The antibodies were ranked with respect to their affinity and selectivity to mutant, denatured and oxidized SOD1. Epitope mapping determined that one region of exon 3 in SOD1 was recognized by several high affinity antibodies. Based on our *in vitro* experiments, 6 antibodies were selected for *in vivo* studies. ELISA results showed that soluble misfolded SOD1 levels rose throughout the majority of the lifetime of the animal before decreasing at end stage, while native levels also increased and remained elevated. Higher molecular weight SOD1 species were apparent after disease onset, which correlated with an increased level of ubiquitination in the extracts. Three antibodies recognized misfolded SOD1 in $SOD1^{G93A}$ mice, but not in non tg mice, by IHC and are now being used to stain ALS patient samples.

Discussion and conclusion: Antibodies were identified that bind with high selectivity to denatured, oxidized, and mutant SOD1 proteins. Antibodies were also used to establish ELISAs to measure misfolded SOD1 in transgenic spinal cord tissue extracts and to visualize misfolded SOD1 in transgenic spinal cord sections by immunohistochemistry.

Acknowledgements: Antibodies were licensed from AviTx Inc., Amorfix Life Sciences and via an MTA from Mass Biologics.

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P274 THE MND-ATTENUATING COMPOUND CUII(ATSM) ACTIVATES THE ANTIOXIDANT NRF2 PATHWAY IN CULTURED ASTROCYTES

LIDDELL J[1], KANNINEN K[2], DUNCAN C[1], KOISTINAHO J[2], CROUCH P[1], WHITE A[1]

[1]*Department of Pathology, University of Melbourne, Parkville, Victoria, Australia,* [2]*A. I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland*

Email address for correspondence: jliddell@unimelb.edu.au

Keywords: therapeutic, Nrf2, astrocyte

Background: We have demonstrated that diacetylbis (4-methylthiosemicarbazonato) Cu^{II} (Cu^{II}(atsm)) significantly delays symptom onset and extends lifespan in multiple transgenic mouse models of motor neuron disease (1, 2, 3).

Objectives: This study seeks to elucidate the mechanism by which Cu^{II}(atsm) induces its potent neuroprotective effects. Nrf2 is an important transcription factor regulating a suite of antioxidant genes and its activation is impaired in MND spinal cord and brain (4). Induction of Nrf2 in MND model mice is protective and astrocyte-dependent (5). Therefore, the protective effects of Cu^{II}(atsm) may involve stimulation of the Nrf2 pathway in astrocytes. In line with this, Cu^{II}(atsm) attenuates oxidative damage and astrocyte activation in MND model mice (2, 3).

Methods: Primary astrocytes cultured from the brains of newborn mice were treated with Cu^{II}(atsm) for up to 24h ($n = 3$-4 independent cultures). Nrf2 activation was assessed by nuclear accumulation of Nrf2 and induction of its targets including heme oxygenase-1 and glutamate-cysteine ligase. The latter controls the synthesis of the critical antioxidant glutathione. Accordingly, cellular and exported glutathione content were also determined. Stimulation of glutathione content and export by Cu^{II}(atsm) was further assessed in astrocytes derived from human neural progenitor cells and in cultured neurons. To exclude non-specific pathways, induction of glutathione was assessed in astrocytes cultured from Nrf2-deficient mice. Increased bioavailability of Cu by Cu^{II}(atsm) was assessed by co-administration with the metal chelator TPEN.

Results: Cu^{II}(atsm) induced nuclear accumulation of Nrf2, increased HO1 expression and GCL activity, and increased glutathione content and export from astrocytes ($p < 0.05$). These effects were replicated in cells of human origin ($p < 0.05$). Cu^{II}(atsm) did not increase the glutathione content or export of cells deficient in Nrf2 ($p > 0.05$), nor in cultured neurons ($p > 0.05$). Co-administration of Cu^{II}(atsm) with the metal chelator TPEN blocked induction of the Nrf2 pathway, including nuclear accumulation of Nrf2, HO1 expression and glutathione content ($p > 0.05$).

Discussion and conclusion: These results demonstrate that Cu^{II}(atsm) activates the antioxidant Nrf2 pathway in cultured astrocytes. These effects appear to translate into human cells, to be limited to astrocytes, and are dependent upon the presence of Nrf2 and increased bioavailable Cu. These actions may contribute to the neuroprotective and disease-attenuating activity of Cu^{II}(atsm) observed *in vivo*, and indicates that Nrf2 may be a valuable therapeutic target for the treatment of motor neuron disease.

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P275 CORRECTING DEFECTIVE ENDOPLASMIC RETICULUM-MITOCHONDRIA INTERACTIONS AS A NEW THERAPEUTIC TARGET FOR ALS: CHARACTERISATION OF NOVEL DRUG SCREENS

MÜLLER S[1], PAILLUSSON S[1], WELSH N[1], STOICA R[1], DE VOS KJ[1, 2], MILLER C[1]

[1]*Departments of Neuroscience and Clinical Neurosciences, Institute of Psychiatry, King's College London, London, UK.* [2]*Department*

of Neuroscience, Sheffield Institute for Translational Neuroscience and Centre for Membrane Interactions and Dynamics, University of Sheffield, Sheffield, UK

Email address for correspondence: sarah.mueller@kcl.ac.uk

Keywords: TDP-43, ER-mitochondria associations, VAPB-PTPIP51 interaction

Background: Mitochondria and the ER form close associations and these regulate a number of fundamental physiological processes including energy and phospholipid metabolism, Ca^{2+} homeostasis, mitochondrial biogenesis and transport, ER stress, autophagy and apoptosis. Disruption of ER-mitochondria contacts has been described in ALS. To dissect the pathological processes involving the ER-mitochondria axis the identification of the molecular tethers that connect regions of ER with mitochondria is essential. Recently, we identified the integral ER protein VAPB and the outer mitochondrial membrane protein PTPIP51 as interacting proteins functioning as tethering scaffolds (1, 2 ,3). Moreover, we showed that expression of wild-type and ALS/FTD mutant TDP-43 disrupts both ER-mitochondria associations and the VAPB-PTPIP51 interaction 1. The VAPB-PTPIP51 interaction thus represents a new therapeutic target for ALS/FTD. Here, we describe a fast and reliable cellular assay for monitoring the VAPB-PTPIP51 interaction which allows screening for small molecules that might correct defective ER-mitochondria and VAPB-PTPIP51 associations in disease.

Methods: We created plasmids in which the cytoplasmic domain of VAPB was fused to the DNA binding domain of the yeast transcription factor GAL4, and the cytoplasmic domain of PTPIP51 was fused to the viral DNA-transactivator domain VP16. HeLa cells were transfected with each of these plasmids either alone or in combination and with a GAL4-UAS luciferase reporter plasmid. Other positive and negative controls were included. We also treated cells with selected kinase inhibitors and monitored their effect on the VAPB-PTPIP51 interaction.

Results: We obtained robust luciferase signals in VAPB-GAL4 (DNA binding domain) and PTPIP51-VP16 co-transfected cells but not in negative control transfected cells. Using the assay and appropriate kinase inhibitors, we identified signalling pathways that impact on the VAPB-PTPIP51 interaction.

Conclusion: We have identified the VAPB-PTPIP51 interaction and ER-mitochondria associations as new molecular targets for the treatment of ALS. We have designed a robust high-throughput screen for identifying small molecules that might correct defective VAPB-PTPIP51 and ER-mitochondria associations in ALS.

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P276 IMPROVEMENTS IN MOTOR FUNCTION, CA2 + CLEARANCE AND MARKERS OF ENDOPLASMIC RETICULUM STRESS WITH 6-GINGEROL TREATMENT IN SOD1^{G93A} ALS MICE

CHIN E, MÁZALA D, CHEN D

University of Maryland, College Park, MD, USA

Email address for correspondence: erchin@umd.edu

Keywords: SERCA function, ER stress, gingerol

Background: We recently identified impairments in intracellular Ca^{2+} clearance, reductions the Sarco/Endoplasmic Reticulum Ca^{2+} ATPase (SERCA) protein expression (SERCA1 and SERCA2) and increased markers of endoplasmic reticulum (ER) stress in skeletal muscle of the SOD1^{G93A} mouse model of ALS. In mouse models of muscular dystrophy there is a rescue of the muscle wasting pathology with overexpression of the fast fibre-specific isoform SERCA1 in skeletal muscle (1). Thus, altered intracellular Ca^{2+} homeostasis may be a common final pathway for mediating cellular death and skeletal muscle atrophy in various neuromuscular diseases.

Objectives: The purpose of this study was to obtain proof of concept for a SERCA activator in improving functional outcomes and rescuing the ER stress associated with skeletal muscle dysfunction in SOD1^{G93A} mice.

Methods: We identified a small molecule, 6-gingerol, which has been shown to increase SERCA1 activity (2). At 35d, mice were assigned to treatment groups: i) wild-type control treated with vehicle (WT-Veh; $n = 4$; 3 female (F) and 1 male (M)); ii) SOD1^{G93A} treated with vehicle (ALS-Tg Veh; $n = 4$; 3 F and 1 M; iii) SOD1^{G93A} mice treated with 6-gingerol (ALS-Tg Gin; $n = 4$; 3 F and 1 M). ALS-Tg Gin mice received 6-gingerol (5 mg/kg, ip) daily for 10 wks; Veh mice received 0.4% ethanol in PBS ip daily.

Results: At 115d, grip function was reduced in ALS-Tg Veh to 17% of WT-Veh level ($p < 0.05$) and showed improvement in ALS-Tg Gin (to 42% of WT-Veh; $p = 0.08$). Intracellular Ca^{2+} regulation was assessed in isolated single muscle fibres using Fura-2. Consistent with our previous studies, resting Fura-2 ratio was significantly increased in ALS-Tg Veh vs. WT-Veh and was lower in ALS-Tg Gin vs. ALS-Tg Veh ($p = 0.13$). SR Ca^{2+} pump function was determined by the time taken for Fura-2 ratio to return to 25% of its baseline level. This Ca^{2+} decay time was measured following 50 and 100 Hz tetani. There was a significant increase in Ca^{2+} decay time in ALS-Tg Veh compared to WT-Veh ($p < 0.05$) and an improvement in ALS-Gin vs. ALS-Veh ($p = 0.11$). Treatment with 6-gingerol rescued the increase in the ER stress-induced cell death marker CHOP and also attenuated the decrease in SERCA1 expression.

Discussion and conclusion: Overall, these data support the premise that 6-gingerol increases intracellular Ca^{2+} clearance by activating the Ca^{2+} pumping function of SERCA, and is associated with reductions of ER stress markers in ALS-Tg mice. These changes are associated with an improvement in neuromuscular function.

Conclusion: These preliminary data provide proof of concept for the use of a SERCA agonist in improving motor function and attenuating the cellular damage that occurs with denervation and muscle atrophy in ALS.

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P277 CNS102 IMPROVES SURVIVAL AND MOTOR BEHAVIOR IN SOD1 MICE AND PROTECTS AGAINST EXCITOTOXICITY THROUGH MULTIPLE SIGNALING PATHWAYS

ERMINI F, PAN Y, BRIDGEWATER J, LISTER H, HAAG WG, ARGADE A, SHIBAMOTO S, SERIZAWA H

Coyote Pharmaceuticals, Menlo Park, CA, United States Minor Outlying Islands

Email address for correspondence: florian.ermini@coyotepharma.com

Keywords: SOD1, heat shock protein, excitotoxicity

Background: CNS102 is the purified all-trans isomer of geranylgeranylacetone (GGA). The isomeric mixture of GGA (teprenone) is a pharmaceutical drug for gastric ulcers in Japan and it has been widely reported to induce the expression of heat shock protein 70 (HSP70). HSP70 is protective in degenerating neurons, and if teprenone is given at an oral dose of 600mg/kg or larger it is neuroprotective in rodent models of neurodegeneration.

Objective: To show that CNS102 is effective against neurodegeneration in rodents at a dose low enough to be feasible for development into a therapeutic treatment of amyotrophic lateral sclerosis (ALS), and to elucidate the pathways through which CNS102 has neuroprotective activity.

Methods: Two rodent models were tested for CNS102 efficacy. $SOD1^{G93A}$ mice (n = 16) were orally administered CNS102 (12 mg/kg), riluzole (8 mg/kg) or vehicle daily starting at P39 and assessed for motor deficits (neurological scores catwalk, grip test, tail suspension test), body weight and survival. Excitotoxic cell death was induced by kainic acid (KA) in Sprague-Dawley rats (n = 9-11) following six daily oral administrations of 100mg/kg CNS102 or PBS. Neuronal cell death was quantified by histology, and HSP expression by ELISA and qPCR. In N2A cells the HSP response to CNS102 was characterized with an i-HSP70 5′-UTR driven luciferase reporter assay, western blot, ELISA and qPCR. Prenylation of the small GTPases Rap1A and RhoA was quantified by western blot and morphological effects were determined with a neurite outgrowth assay.

Results: CNS102-treated SOD1 mice performed better on the cat walk demonstrating improved stride length and running speed. Grip strength and hind leg extension by tail hang assay was improved, and neuroscore analysis showed that the rate of increase was 33% greater in vehicle- vs. CNS102-treated SOD1- mice. Physiologically, CNS102 reduced the rate of loss of body weight by over 40% compared to vehicle-treated SOD1 mice. Median survival was prolonged by 8 days ($p < 0.0007$) and 6.5 days ($p < 0.015$) in CNS102 and riluzole treated SOD1 mice respectively, compared to vehicle treated SOD1 mice. In rats CNS102 reduced excitotoxicity induced neuron loss by 33% and increased HSP70, GRP78 and HSP27 expression. In N2A cells we found a dose response to CNS 102 of HSF1, HSP70, HSP40 and HSP90, increased prenylation of Rap1A and RhoA and stronger growth of neurites.

Discussion and conclusion: We demonstrate efficacy of CNS102 for improvements in survival and motor deficits in SOD1 mice, and protection against excitotoxic neurodegeneration in rats. Our data suggests that the neuroprotective effect of CNS102 might be mediated through modulation of the HSP response and prenylation of small GTPases. The low effective dose in the SOD1 model makes CNS102 a feasible candidate for development as a therapeutic for ALS.

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P278 INTRATHECAL BACLOFEN FOR SPASTICITY IN MOTOR NEURON DISEASE (MND): LONG-TERM EXPERIENCES

SCHWEIKERT K[1,2], WILMES S[1], KÄTTERER C[1], WASNER M[3], NEUWIRTH C[4], WEBER M[4]

[1]*REHAB, Basel, Switzerland,* [2]*Neuromuscular Center, University Hospital, Basel, Switzerland,* [3]*Neurosurgery, University Hospital, Basel, Switzerland,* [4]*Muscular Disease Unit/ALS Clinics, St. Gallen, Switzerland*

Email address for correspondence: schweikert.k@gmx.net

Keywords: intrathecal baclofen (ITB), spasticity, long-term-follow-up

Background: Management of severe spasticity in MND is often unsatisfactory due to intolerance or inefficacy of oral medications. In patients with primary lateral sclerosis (PLS) and upper-motor neuron predominant ALS, intrathecal baclofen (ITB) therapy can be an option. However, little is known about long-term outcome in these patients.

Objectives: To report on long-term experiences with ITB for severe spasticity in MND patients in Switzerland.

Methods: A total of 16 patients, referred by ALS clinics for evaluation of ITB therapy, were examined by a neurologist, an occupational, a speech, and a physiotherapist at baseline. In all patients, ITB was administered by a probatory external pump (connected with a subcutaneous intrathecal catheter about 40–60 cm above L3/L4 puncture level), the dosage was increased according to clinical signs and oral antispastic medication tapered off and stopped. ALS Functional Rating Scale (ALSFRS-R), Functional Independence Measure scores, speech, swallowing, and spasticity (modified Ashworth scale) were evaluated before and under ITB therapy. Only in case of clear benefit, was a permanent ITB pump implanted. All patients were followed in ALS clinics.

Results: From 2/2007 to 5/2014, sixteen patients (12 men, 4 women), mean age 48.5 years, were treated with ITB via probatory external pump. Four patients were diagnosed with PLS, 12 with ALS. At baseline, mean disease duration was 55 months, ALSFRS-R 29.2. In all patients spasticity was reduced, no side effects occurred. Four patients did not go on a permanent ITB pump because symptoms did not improve or deteriorated. A permanent pump (Synchromed II, Medtronic) was implanted in 12 patients, mean ITB starting dosage 50 ug/d. All patients, followed in ALS clinics (one lost to follow-up), continued ITB therapy. Seven patients died of respiratory failure due to progression of MND. In this group, mean duration of ITB treatment was 18.1 months, compared to 29.5 months in the 4 patients who are still alive. At the last evaluation, mean ALSFRS-R was 11, and 27.5, ITB dosage 70 ug/d, and 135 ug/d respectively.

Discussion and conclusion: In MND patients, the pattern of muscle tone and strength varies substantially and individually. Severe spasticity might require ITB therapy, but progression of atrophic paresis has to be considered. In our patients, escalation of ITB dosage in the course of the disease was often needed.

Conclusion: ITB can safely and effectively reduce spasticity in long-term course of selected patients with MND.

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P279 PSYCHOLOGICAL AND PSYCHOTHERAPEUTIC APPROACHES FOR PEOPLE WITH MND: A QUALITATIVE STUDY

RABBITTE M[2, 1], BATES U[2, 1], KEANE M[1]

[1]*Dublin City University, Dublin, Ireland,* [2]*Our Lady's Hospice Care Services, Blackrock, Ireland*

Email address for correspondence: mary.rabbitte2@mail.dcu.ie

Keywords: psychological, psychotherapeutic, palliative care

Background: People with MND are often dissatisfied with the level of care provided, as they move their focus from physical health concerns to emotional support, as the disease progresses (1). However there seems to be little or no research on the range and effectiveness of psychotherapeutic interventions that could support the person with MND.

Objective: The aim was to review current psychotherapeutic approaches used by counsellors, psychologists and psychotherapists in Ireland and gain an insight into approaches used elsewhere.

Methods: A qualitative study was used using semi-structured interviews. Participants include 8 Irish therapists representing public and private service and 2 therapists from UK and Italy representing public service. Data was analysed using principles of grounded theory (2) to generate principal categories that best describe the therapists' approaches. The interview schedule was designed in collaboration with a person with a terminal neurological disorder.

Results: From this study five principal categories were identified that outline the therapists' approaches as follows: Therapy pre-requisites (creating a therapeutic space in an appropriate timeframe and location); Experience and awareness of MND (understanding the physical and possible cognitive impacts of MND); Relationship context (embracing the emotional affect for the person with MND by managing own emotional affect);Theoretical model and interventions (offering a combination of supportive and empowering approaches); Perceptions of outcome (providing space to talk and express feelings, self-direct and ease them on their journey).

Discussion and conclusion: There is no consensus about a specific approach; due to the complexity of the disease and variety of presentations. Common approaches included supporting the person in the "here and now" by providing a "fine focus" on what they can still do, re-affirming their ability to self-direct and supporting emotional exploration. The findings indicate therapists should have an experience of MND, the limitations in mobility, communication and cognitive processing, to be able to provide a safe place to talk.

Therapists use various approaches that provide different perceived outcomes. Different approaches may be required during disease progression. Therapists need to understand the pre-requisites and have experience of MND. Therapists' awareness and management of their own emotional affect enhances their therapeutic ability.

Acknowledgements: The participants. Professor Orla Hardiman, Bernie Corr and Irish Motor Neurone Disease Association (IMNDA) for support in recruitment. Irish Hospice Foundation for sponsoring a development grant for psychosocial training based in part on this research.

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P280 GENDER DIFFERENCES IN EMERGING BEHAVIORAL CHANGE IN ALS SUGGEST A NEUROENDOCRINE MODEL FOR TREATMENT

FLAHERTY C[1], BROTHERS A[2], VANKAMPEN D[3], ZANGENEH SADEGHI A[1], YANG C[1], LEGRO R[1], MANNI A[1], SIMMONS Z[1]

[1]*Penn State College of Medicine, Hershey, PA, USA,* [2]*Colorado State University, Fort Collins, CO, USA,* [3]*Penn State University, Middletown, PA, USA*

Email address for correspondence: cflahertycraig@hmc.psu.edu

Keywords: gender, behaviour, neuroendocrine

Background: ALS is associated with frontotemporal lobar degeneration (FTLD) in ~50% of patients, in the absence of dementia, characterized by primary progressive aphasia and/or behavioural decline. Our published work demonstrated gender differences in ALS with behavioural impairment (ALSbi) that included significantly more males with the Disinhibited subtype, including impulsivity, jocularity, and loss of insight. The Apathy subtype was equivalent between genders, while a significantly greater proportion of women evidenced Personal Neglect. From these findings we generated a theory of FTLD emergence involving midbrain dominance and motivation circuitry. In conjunction with this, we hypothesize a neuroendocrine model of neuroprotection whereby oestrogen replacement may forestall disease progression in peri-menopausal female ALS patients with emerging signs of FTLD.

Objectives: We investigated gender differences in pattern of emergence of behavioural change in ALS, and their relationship to oestrogen levels in females.

Methods: Behavioural assessment was pursued by evaluation of patient executive functioning and caregiver interview with the structured Frontal Behavioural Inventory (n = 171). To evaluate executive functioning, we assessed patients with the Penn State Brief Exam of Frontal and Temporal Dysfunction Syndromes

Results: Consistent with our previous findings, we found a significantly greater number of males with the Disinhibition subtype ($p = 0.014$). Males also showed a greater proportion of the Stereopathy behavioural subtype ($p = 0.048$). Apathy was again equivalent between genders, while females again evidenced a greater incidence rate of moderate-severe Personal Neglect (7.0%) in comparison to males (4.5%). Medication records review of female estrogen status for patients aged 31-74, including oestrogen replacement in peri-menopausal and menopausal patients, showed a strong relationship to both higher executive functioning capacities (similarities ($p = 0.005$), judgment ($p = 0.018$), letter fluency ($p = 0.004$)), and attenuation of Apathy ($p = 0.022$), the latter unrelated to age ($p = 0.076$).

Discussion and conclusion: Gender differences are present in emerging ALSbi, and relate to oestrogen status. These findings evidence the potential of oestrogen as a therapeutic agent to attenuate executive functioning and behavioural decline in emerging ALS-FTLD. Given the overlap in genes associated with ALS, FTLD and breast cancer (1), as well as the conflicting findings in literature on the benefit and risks of oestrogen replacement, gonadal steroidal hormones likely serve as immuno-modulatory agents. Their action may range from inhibitory to stimulatory in a concentration dependent manner, influenced as well by the nature of the target tissue. Oestrogen analogs are needed to attenuate neurodegeneration while inhibiting over-activation in the breast and uterus, akin to the selective oestrogen receptor modulators currently applied as therapeutics in the treatment of breast cancer (2).

Acknowledgements: We thank our patients for their dedication and commitment.

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P281 IMPACT OF EXPIRATORY MUSCLE STRENGTH TRAINING ON BULBAR FUNCTION IN AMYOTROPHIC LATERAL SCLEROSIS: UPDATES FROM A RANDOMIZED SHAM-CONTROLLED CLINICAL TRIAL

PLOWMAN EK, ROSADO M, TABOR L, TURNER K, GAZIANO J, RICHTER J, GOOCH C, VU T

University of South Florida, Tampa, Florida, USA

Email address for correspondence: plowman@usf.edu

Keywords: exercise, treatment, expiratory muscle strength training (EMST)

Background: The role of exercise in individuals with ALS is controversial. We have recently reported that expiratory muscle strength training (EMST) is feasible, safe and lead to improvements in expiratory force generating pressures, swallowing kinematics, cough spirometry and airway protection during swallowing in a pilot study of 25 ALS patients. Further work is needed to validate these preliminary findings and to further elucidate the potential role of exercise in this patient population.

Objective: Determine the efficacy of a targeted bulbar strength training program (EMST) on maximum expiratory pressure (MEP), swallow kinematics, cough spirometry, quality of life and disease progression in ALS patients with mild to moderately severe symptoms.

Methods: This is a randomized blinded sham-controlled clinical trial enrolling 48 patients with mild-moderate ALS (possible, probable or definite Revised El-Escorial Criteria). Patients will undergo eight-weeks of daily training with an active ($n = 24$) or sham ($n = 24$) device. The primary outcome variable is MEP (cmH_2O). Secondary measures include: kinematic and temporal swallowing indices; cough spirometry measures; and the Penetration Aspiration Scale score (an index of airway safety during swallowing). Tertiary outcomes include patient-reported measures of: swallow-related quality of life; dysphagia severity; and functional oral intake using validated scales (SWAL-QOL, EAT-10, FOIS respectively). Finally, the impact of EMST on disease progression over time will be investigated via the ALSFRS-R. Statistical analysis performed on interim data constituted a 2×2 (time $\times$ group) mixed model ANOVA with alpha set at 0.05.

Discussion and conclusion: At time of abstract submission, 28 individuals have been enrolled in this RCT with 20 individuals completing the trial. Interim data indicate a significant time by group interaction for the primary outcome variable, Maximum Expiratory Pressure ($F(1) = 9.10$, $p = 0.01$). Post-hoc analysis revealed a significant increase in MEPs for ALS patients in the active EMST group ($p = 0.03$, mean difference $37.35 cmH_2O$) and a significant between groups difference (active vs. sham) at the post-treatment time point ($p = 0.02$, mean difference $78.45 cmH_2O$). A significant group by condition interaction was also revealed for ALSFRS-R scores. Post-hoc analysis revealed a significant reduction for the sham group pre vs. post-treatment ($p = 0.02$) but not for the active group ($p = 0.47$). No significant differences were revealed for patient-reported swallowing severity data (EAT-10), however those in the active group had a mean group improvement of 42.02% while those in the sham group demonstrated a 1.36% improvement. Cough spirometry and kinematic swallow physiology measures are currently being analyzed. These data and those of currently active patients enrolled in this trial will be presented at the 25th International Symposium on ALS/MND.

Current interim data from this RCT confirm our previous findings and suggest that strength training of bulbar musculature may be beneficial for improving and maintaining expiratory generating pressures and may impact measures of global disease progression.

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P282 MECHANICAL INSUFFLATION/EXSUFFLATION WITH HIGH FREQUENCY CHEST WALL OSCILLATION: RESULTS OF A CLINICAL TRIAL

ROSENFELD J[1,2], MARQUEZ I[1,2]

[1]*ucsf, Fresno, California, USA,* [2]*Central California Neuroscience Institute, Fresno, California, USA*

Email address for correspondence: jrosenfeld@fresno.ucsf.edu

Keywords: respiratory management, therapy, standards of care

Background: Secretion management is an essential aspect of respiratory care in patients with motor neuron disease. Poor or compromised muscle strength can eventually lead to atelectasis, and infection, especially in the context of impaired lung volumes. Mechanical Insufflation/Exsufflation (I/E) has been highly effective in assisting with mobilization of secre-

tions by mimicking a cough reflex and in the delivery of high inspiratory volumes. Cycling between insufflation and exsufflation can either be performed manually or automatically on the coffalator unit.

Patients with poor mucocilliary action may develop excessive secretions blocking the smaller airways that may not be easily mobilized with mechanical I/E therapy. With the smaller airways, The Vest or Respirtech therapy unit is based upon a technology called high-frequency chest wall oscillation (HFCWO). HFCWO therapy is administered by a device consisting of an inflatable vest connected by hoses to an air-pulse generator. The generator rapidly inflates and deflates the vest, gently compressing and releasing the chest wall to create airflow within the lungs. Evenly distributed oscillating forces applied externally to the chest wall generate cough-like shear forces within the airways that dislodge mucus from the bronchial walls, increase mobilization, and move it along towards central airways. This action also works to thin thick secretions, making them easier to clear. Once the mucus has advanced from smaller to larger airways, it can be easily removed by coughing and expectoration or by suctioning.

We have recently completed a clinical trial, comparing I/E therapy with the combination of I/E therapy and HFCWO.

Methods: Patients completed a battery of pulmonary function studies, body plethysmography, CT scan of the chest and respiratory function questionnaires. Lung volume, pre and post treatments will be compared as well as the benefit to patient's ventilation and respiration.

Results: Results of our final analysis will be presented. Currently, the last of the patients enrolled are completing the study and all data will be available by the time of the presentation.

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P283 RELATIONSHIPS BETWEEN RILUZOLE AND TIRASEMTIV LEVELS ON OUTCOMES IN THE BENEFIT-ALS TRIAL

SHEFNER J[1], ANDREWS J[2], MENG L[2], BIAN A[2], WOLFF A[2], STUDY GROUP BENEFIT ALS[2]

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

Email address for correspondence: jmshefner@gmail.com

Keywords: clinical trials, skeletal muscle activation, pharmacodynamics

Objectives: The BENEFIT-ALS Trial evaluated the effects of Tirasemtiv, a fast skeletal muscle activator, in 711 patients with ALS randomized either to placebo or to an escalating dose of Tirasemtiv up to 500 mg per day. After 3 months, statistically significant differences favouring Tirasemtiv were found in extremity strength and in slow vital capacity (SVC), although there was no significant difference between groups in ALSFRS-R. In this report, we examine the relationship between plasma Tirasemtiv levels and riluzole levels on patient efficacy outcomes and adverse events.

Methods: 711 patients enrolled in the study; 106 patients dropped out prior to randomization and 156 patients were removed from the analysis due to a drug dispensing error that occurred mid-study. All other patients who had at least 1 efficacy assessment are included in this report. At each study visit, both plasma riluzole and Tirasemtiv levels were obtained. The relationship of adverse event frequency to both riluzole and Tirasemtiv levels was determined combining all visits. For all efficacy measures (ALSFRS-R, SVC, Maximum Voluntary Ventilation (MVV), Sniff Nasal Inspiratory Pressure (SNIP), Muscle Strength assessed via Hand Held Dynamometry (HHD)), patients were divided into concentration quartiles for both riluzole and Tirasemtiv, and analyses based on slope of percent change from baseline were performed.

Results: Although riluzole levels in the Tirasemtiv group were approximately 40% higher than in the placebo group, there was no indication that this difference was related to frequency or intensity of adverse events. The relationship between treatment emergent adverse events and Tirasemtiv levels were evaluated. Efficacy measures were evaluated both as a function of maximum tolerated Tirasemtiv dose and serum Tirasemtiv concentration. For SVC, there was a positive relationship between serum concentration and SVC benefit. Initial modelling suggests that the relationships between Tirasemtiv concentration and other efficacy measures were not linear. With respect to efficacy, riluzole levels had no impact on any measure.

Discussion and conclusion: In the BENEFIT-ALS trial, riluzole seemed to not have an impact either on Tirasemtiv efficacy or tolerability, suggesting that the strategy of dose lowering in patients on Tirasemtiv had the intended effect. Adverse events were evaluated with respect to Tirasemtiv levels; the relationship of Tirasemtiv to measures of efficacy was complex. Multiple models relating Tirasemtiv concentration to efficacy measures will be explored and presented. These results will help inform further development of this agent.

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P284 EFFECT OF RILUZOLE TREATMENT IN PATIENTS FROM EMILIA ROMAGNA, ITALY: A POPULATION BASED STUDY

MANDRIOLI J[1], FINI N[1], GEORGOULOPOULOU E[1], DE PASQUA S[2], D'ALESSANDRO R[3]

[1]*Department of Neuroscience, St. Agostino-Estense Hospital, MODENA, Italy,* [2]*Department of Neuroscience, University of Parma, PARMA, Italy,* [3]*Neuroepidemiology Unit, IRCCS Istituto delle Scienze Neurologiche di Bologna, BOLOGNA, Italy*

Email address for correspondence: j.mandrioli@ausl.mo.it

Keywords: riluzole, population based registry, survival

Background: Riluzole is the only drug approved for ALS treatment. Although, a number of concerns about effectiveness still persist, mainly due to the slight increase in survival in front of a relatively high cost of the drug. On the other hand we do not have other pharmacological therapeutic strategies, so the drug is approved in many western countries, and largely used in Europe and in Italy.

Methods: This study was performed in 9 provinces and 11 local health units of Emilia Romagna (population 4.4 million inhabitants), with the involvement of 17 neurological departments. From 2009 onwards, a prospective registry has been collecting all cases of incident ALS among residents in Emilia Romagna region. For each patient, the main demographic and clinical information were collected by the caring physicians. In addiction a follow up case report form has been completed

during each patient follow up reporting data on support procedures, death, and information on treatments and their interruption.

Results: From 1.1.2009 to 31.12.2013 in Emilia Romagna, 566 patients (54.9% M, 45.1% F) received a new diagnosis of ALS. Mean time from onset to diagnosis was 12.9 months. Mean age at onset was 66.4 years. 470 patients (83%) were treated with riluzole, whereas 96 patients were not. Median overall tracheostomy-free survival was 50 months (from onset). Patients who did not take Riluzole were older (mean years at onset: 69.8 years vs 65.7 years, $p < 0.01$), were more frequently bulbar ($p = 0.01$) and more frequently demented ($p = 0.02$). Moreover, patients who did not take riluzole were more frequently classified as possible ALS according to El-Escorial Diagnostic criteria (EEC) at diagnosis.

Overall, riluzole treatment did not influence the rate of tracheostomy-free survival of patients. This was confirmed at stratified analysis, which showed that riluzole treatment did not prolong survival in any of the examined subgroup (bulbar, younger patients, definite or probable ALS according to EEC).

Discussion and conclusion: This is an observational study on the use of riluzole in ALS patients from an Italian registry. When the first RCT on riluzole showed benefit from the treatment many concerns were raised, mainly due to the greater benefit in bulbar patients. This led to further RCTs, among which one, including also advanced ALS, did not demonstrate an effect of the drug on survival. The same results were reported after a fourth trial carried out in Japan. Our study has many limitations due to its observational nature, but shows that riluzole treatment does not change survival significantly in a population based setting.

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P285 DISCONTINUOUS RILUZOLE TREATMENT MAY PROVIDE A BETTER THERAPEUTIC ALTERNATIVE FOR ALS PATIENTS

DENNYS C, LEVY M, FRANCO MC, FERNÁNDEZ-VALLE C, ESTÉVEZ A

University of Central Florida, Orlando, Florida, USA

Email address for correspondence: cdennys@knights.ucf.edu

Keywords: riluzole, glial cells, neurotrophic factors

Background: Riluzole is the only FDA approved drug for the treatment of amyotrophic lateral sclerosis. The mechanism by which riluzole affords its protection is unknown. We have previously shown that riluzole stimulates astrocytes to produce trophic factors for motor neurons.

Objective: We hypothesize that the protective effect of riluzole is due to stimulation of trophic factors by motor neuron associated cells.

Methods: *In vitro* studies*:* Astrocytes were treated with 1μM riluzole for 24 hours and 6 days. Schwann cells were treated with 1μM Riluzole for 24 hours and 3 days. Riluzole was removed before allowing the cells to condition new media for 24 hours. Purified motor neurons were cultured for three days in a 1:10 dilution of the conditioned media. I*n vivo* studies*:* Nontransgenic mice were treated with 100mg/mL riluzole in the drinking water for 3, 6, 15 and 30 days. Animals were then sacrificed and muscle, brain, spinal cord and sciatic nerve were removed. Trophic factor levels were quantified using ELISA and qPCR.

Results: Conditioned media from astrocytes treated with riluzole for 24 hours increased motor neuron survival when compared to untreated conditioned media. Neutralizing antibodies against cardiotrophin-1 (CT-1) partially blocked the trophic factor support following 24 hours riluzole treatment ($p < 0.005$). Similar results were obtained using Schwann cells treated with riluzole for 24 hours ($p < 0.005$). Conditioned media from cells with chronic riluzole treatment did not increase motor neuron survival in comparison to untreated controls. CT-1 expression in Schwann cells was induced following 24 hours riluzole treatment but decreased significantly at 6 days ($p > 0.05$). The MAP kinase p38, a critical regulator of Schwann cell differentiation and myelination, is inhibited following 24 hours riluzole treatment. Cardiotrophin-1 expression in Schwann cells was induced by inhibition of p38. In mice treated with riluzole, there is an increase in the production of GDNF, BDNF, and CT-1 in the spinal cord during the first 15 days, but at 30 days the level of these trophic factors was reduced significantly. The same effect was observed in muscle at 15 days. CT-1 production in the sciatic nerve increased by 6 days, but decreased significantly at 15 days ($p < 0.05$). Expression levels of GDNF, BDNF and CT-1 mRNA followed similar trends than protein levels as determined by qPCR.

Discussion and conclusion: The results reveal that chronic riluzole treatment reduces trophic factor production by glial cells. These results suggest that riluzole has opposite acute and chronic effects on the production of trophic factors, which can explain the small protective effect on ALS patient survival.

Adjusting the dosing regimen for ALS patients may improve drug efficacy and patient survival.

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P286 FAST SKELETAL MUSCLE TROPONIN ACTIVATOR TIRASEMTIV INCREASES MUSCLE FUNCTION AND PERFORMANCE IN MOUSE MODELS OF SPINAL MUSCULAR ATROPHY

HWEE DT[1], KIM L[1], REEDICH E[2], ARNOLD WD[3], KUNTZ NL[2], DIDONATO CJ[2], MALIK FI[1], JASPER JR[1]

[1]*Cytokinetics, Inc., South San Francisco, CA, USA,* [2]*Lurie Children's Hospital of Chicago, Chicago, IL, USA,* [3]*The Ohio State University, Columbus, OH, USA*

Email address for correspondence: dhwee@cytokinetics.com

Keywords: SMA, muscle atrophy, fast skeletal troponin activator

Background: The small molecule *Tirasemtiv* is a specific fast skeletal muscle troponin activator that sensitizes the sarcomere to calcium, leading to increased muscle force *in situ* in response to sub-maximal rates of nerve stimulation and decreased fatigability.

Objectives: The objective of this study was to investigate the effect of *Tirasemtiv* on skeletal muscle function in two SMA mouse models with mild and moderate levels of muscle dysfunction and weakness.

Methods: Two SMA mouse models were evaluated: a model corresponding to intermediate SMA and a less severe model corresponding to adult-onset SMA. Both models were evaluated *in situ* for plantarflexor isometric muscle force in response

to sciatic nerve stimulation, *in situ* muscle fatigability, and *in vivo* forelimb grip strength and inverted grid hang time.

Results: Intermediate and adult onset SMA mice had lower compound muscle action potentials (CMAP), motor unit number estimation (MUNE) numbers, and hindlimb muscle atrophy. Compared to sibling controls (CON), isometric muscle force *in situ* was significantly lower in both SMA mouse models at all submaximal and tetanic rates of nerve stimulation (10 to 200Hz) ($n = 10$-15/group, $p < 0.0001$, CON vs. SMA). In the intermediate SMA mice, *Tirasemtiv* (10 mg/kg, IP) significantly increased isometric force in response to submaximal (20Hz) nerve stimulation in both female (Vehicle:37 ± 4.7mN vs. *Tirasemtiv*:62 ± 7.2mN, mean $\pm$ S.E.M., $n = 6$/group, $p < 0.05$) and male (Vehicle:24 ± 4 mN vs. *Tirasemtiv*:47 ± 7.9 mN, $n = 4$-5/group, $p < 0.05$) SMA mice. In adult onset SMA mice, *Tirasemtiv* (10 mg/kg, IP) significantly increased submaximal isometric force in response to nerve stimulations between 10-60 Hz ($n = 7$-8/group, $p < 0.001$). In both mouse models, *Tirasemtiv*-treated SMA mice had higher muscle force under fatiguing conditions induced by repeated nerve stimulation. *Tirasemtiv* (10 mg/kg, PO) significantly increased forelimb grip strength *in vivo* in intermediate SMA mice compared to vehicle (43 ± 3.8g vs. 52 ± 4.4g, $n = 9$/group, $p < 0.05$). Adult-onset SMA mice had significantly lower hang time *in vivo* compared to CON mice (CON:197 ± 23 sec, $n = 17$ vs. SMA:138 ± 18 sec, $n = 25$, $p < 0.05$). *Tirasemtiv*(10 mg/kg, PO) significantly increased inverted grid hang time in SMA mice (138 ± 18 vs. 192 ± 34 sec, $n = 25$, $p < 0.05$).

Discussion and conclusion: Intermediate and adult-onset SMA mice exhibited nerve dysfunction, muscle atrophy, and weakness. Single doses of *Tirasemtiv* significantly increased submaximal force and fatigue resistance *in situ*, and grip strength and grid hang time *in vivo* in SMA mice. These results suggest that *Tirasemtiv* and other fast skeletal muscle troponin activators may be viable therapeutics for improving muscle function in spinal muscular atrophy.

Acknowledgements: Generation of the SMA mice was funded by NIH and Families of Spinal Muscular Atrophy (FSMA). Conduct of the studies with *Tirasemtiv* was funded by FSMA and Cytokinetics, Inc. DTH, LK, FIM, and JRJ are currently employees of Cytokinetics, Inc. and were compensated financially for their work.

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P287 DIPALS: PATIENT AND CARER EXPERIENCES OF DIAPHRAGM PACING IN MOTOR NEURONE DISEASE

MCDERMOTT C AND THE DIPALS STUDY GROUP[1], BENTLEY A[2], STRADLING J[3], ACKROYD R[4], BAUDOUIN S[5], MORRISON K[6], MUSTFA N[7], MAYNARD N[3], GALLOWAY S[8], KARAT D[5]

[1]*University of Sheffield, Sheffield, UK,* [2]*Wythenshawe Hospital, Oxford, UK,* [3]*Churchill Hospital, Oxford, UK,* [4]*Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, UK,* [5]*Royal Victoria Infirmary, Newcastle Upon Tyne, UK,* [6]*University of Birmingham, Birmingham, UK,* [7]*University Hospital of North Staffordshire, Stoke on Trent, UK,* [8]*University Hospital of South Manchester, Manchester, UK*

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

Keywords: diaphragm pacing, respiratory support, patient views

Background: Diaphragm movement is an essential element of adequate respiration. There has been considerable interest therefore in using methods of diaphragm stimulation to maintain respiratory function in patients with motor neurone disease (1). Evidence of its effectiveness is limited however, and there have been suggestions that some patients may find it difficult to tolerate (2).

Objectives: The qualitative work reported here forms part of a large-scale randomised controlled trial of the NeuRx RA/4 Diaphragm Pacing System in patients with motor neurone disease (ISRCTN53817913). Objectives of this component are: i) to evaluate the acceptability of the pacing device; ii) to explore the impact of having the system fitted on everyday living.

Methods: Data were collected at two time points, at one month following surgery and six months later. Semi-structured interviews were carried out with patients and carers in their homes.

Results: Fourteen patients took part in the qualitative element of the study. Nine were able to be interviewed at both time points. Participants described the journey from initial invitation to take part in the study, expectations of the procedure versus the reality, post-operative care, views and operation of the equipment, impact on everyday activities, perceptions of the effect, to reflections in hindsight on having the pacer fitted. Patient experience of the surgery varied considerably, with a number having post-surgical complications and longer than expected length of stay, whereas others described it as a minor procedure. Operation of the equipment was generally described as uncomplicated, although some had found the positioning of the socket to be inconvenient, and the fragility of the wiring was reported to be a concern. There was considerable variation in patient ability to tolerate the sensation of the pacer working. At follow up, all patients still had the system in operation with usage varying from two hours to 24 hours per day. The system was rated positively compared to non-invasive ventilation (NIV) however participants described uncertainty regarding any perceived benefit from using the device.

Conclusion: Patient experiences of trialling diaphragm pacing varied considerably. While some experienced pain using the device, others reported feeling only a minor sensation. The device was generally acceptable to patients in terms of ease of operation and impact on life, and while not perceiving immediate gains compared to NIV, patients described their hopes for a beneficial effect in the long term.

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P288 PRO-ACT: EARLY RESULTS FROM THE LARGEST ALS CLINICAL TRIALS DATABASE

ALON H[1], ZACH N[1], SHERMAN A[2], WALKER J[2], SINANI E[2], KATSOVSKIY I[2], SHUI A[3], SCHOENFELD D[3], KUEFFNER R[4], STOLOVITZKY G[5], NOREL R[5], ATASSI N[2], BERRY J[2], CUDKOWICZ M[2], LEITNER M[1]

[1]*Prize4Life, Tel Aviv, Israel,* [2]*Neurological Clinical Research Institute, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA, USA,* [3]*MGH Biostatistics Center, Massachusetts General Hospital, Boston, MA, USA,* [4]*Ludwig-Maximilians University, Munich, Germany,* [5]*IBM T.J. Watson Research Center, Yorktown Heights, NY, USA*

Email address for correspondence: alonh@prize4life.org

Keywords: disease progression, patients stratification, PRO-ACT

Background and objectives: Large datasets are critical for identifying statistically significant and emphasize biologically relevant observations, especially in a rare disease like ALS. The heterogeneity of the ALS patient population presents a substantial barrier to understanding disease mechanisms and to the planning and interpretation of ALS clinical trials, leading to large, expensive, and potentially unbalanced trials. Therefore, pooling together information from completed ALS clinical trials - into the Pooled Resource Open-access ALS Clinical Trials (PRO-ACT) platform provides an unprecedented opportunity to increase our understanding of the ALS patient population and further ALS research. In this presentation we will outline results already gained from analyses performed on the PRO-ACT database.

Methods and results: The PRO-ACT platform contains the records of over 8500 ALS patients who participated in 17 completed clinical trials. Data include demographics, family history, vital signs, clinical assessments, lab data, medication and survival information, as well as newly added information about concomitant medication use and adverse events. Additional data are expected to be introduced to the database in 2015. The database was made open-access to researchers worldwide in December, 2012, and since then has attracted the attention of over 330 researchers from 42 countries, including 26 pharmaceutical companies and over 100 academic institutions and hospitals.

The PRO-ACT database provides the unique and unprecedented opportunity to gain deeper understanding of the natural history of the disease, to estimate various traits including individual disease progression and survival, to allow patient stratification and help identify potential diagnostic markers for disease progression. Indeed, analysis of the PRO-ACT database has revealed several novel and important findings, including: identification of several baseline variables that significantly correlate with ALSFRS slope in a multivariate analysis (controlling for age, gender, time from onset and baseline functional measures); Development of novel methods to stratify patients into slow and fast progressors; Identification of several novel predictors of disease progression and of survival and of factors differentiating specifically the very fast and very slow patients.

Discussion and conclusion: These results demonstrate some recent advances in our understanding of ALS clinical information, as well as the power of the PRO-ACT database in addressing important yet unaddressed questions. They demonstrate the importance of pooling together large amount of information for developing a better understanding of ALS natural history, prognostic factors and disease variables and patient stratification. These finding, and future findings to come, can foster our understanding of ALS, and be of help in the design of future clinical trials.

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P289 PHYSICIANS, RESEARCHERS AND PATIENTS AS WILLING PARTICIPANTS IN CLINICAL RESEARCH ENTERPRISE: INCENTIVES AND TECHNOLOGY

SHERMAN A[1], KATZ J[2], CUDKOWICZ M[1]

[1]*Massachusetts General Hospital, Boston, MA, USA,* [2]*CPMC, San Francisco, CA, USA*

Email address for correspondence: avsherman@partners.org

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Background: It is beneficial for medical, research and patient communities to aggregate and cross-link clinical and research information from clinical encounters, clinical studies, health records, and self-reported patient outcomes, while connecting to biospecimen and image repositories. Absence of incentives and desire to share and collaborate slows progress. Patient empowerment is a new paradigm.

Objective: To introduce system of incentives and supporting technologies for standardized yet flexible approach to secure collaboration, integration, harmonization and sharing of clinical and research information by all clinical research enterprise participants.

Methods: Patients, patient advocacy groups, clinicians and researchers were interviewed on foundations that incentivize them to collaborate and share information (physicians/researchers) and participate in research (patients/caregivers/advocacies). Results of surveys and recommendations from PCORI taskforces are analyzed and applied to NeuroBANKTM as truly collaborative platform for disease-specific clinical research. NIH-developed Global Unique ID (GUID) technology allows linking various data sources into coherent distributed meta-dataset, while maintaining regulatory compliance. Disease-area-specific central authority for generating GUIDs is set up by Neurological Clinical Research Institute to assist the neurodegenerative diseases clinical and research community with collecting patients' data and linking it to other information. Informed Consent Metalayer tracks and matches information with its requestor.

Results: For clinicians/researchers, the major obstacles quoted were absence of time, of funding to support data entry process, and too many obligations to fulfil. Some of these issues could be resolved with a carrot/stick approach: additional funding ("carrot", Canadian ALS Association), threat to withdraw existing funding ("stick", MDA, NIMH) or combination (MDA). Technology help with a single point-of-entry approach, as data are captured once and system distributes cleaned data further according to physicians' obligations (EHR, disease registries, hospital repositories, etc.) in predefined formats. Additional incentives are generated by the system patients' summary reports and post-patient-encounter reports. Utilization of existing records with ability to enter new information is paramount. Virtual biobanking approach with centralized clinical data repository proved to be successful and sensible.

From patients' perspective, incentives to participate in research vary with patients' age and disease progression. While

survival concerns prevail, quality of life is more important in the adult population. Current trends suggest shift of power in clinical research enterprise towards patients, especially in data sharing, patient-reported outcomes and patients' control of research directions. Patients may refuse to participate in research projects if information is not shared freely.

Conclusion: NeuroBANKTM platform allows aggregation of existing datasets and direct data capture. Integration of the GUID technology with NeuroBANKTM provides mechanism of linking same-patients' data from multiple sources. GUID technology is uniquely suitable for use with de-identified datasets. It may link biospecimen collections and images with clinical and research data and electronic health records. GUID approach facilitates international collaboration, even when without affiliation with NeuroBANKTM and other collaborative efforts.

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