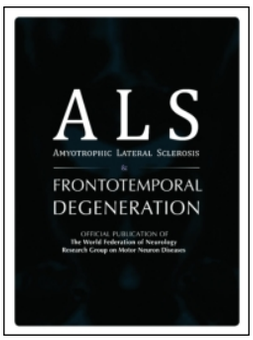

Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration

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Sessions 1 - 11

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Session 1 Joint Opening Session

C1 INSIGHTS INTO THE ALS/MND EXPOSOME27th International Symposium on ALS/MND

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Keywords: exposome, modifiable risk factors, OMICs

Major technological advancements in the last decade have resulted in extensive new insights in the role of genomics and biology in health. While these advances are impressive, they have not necessarily translated into large-scale public health improvements. At the same time, there has been increasing recognition of the crucial and significant role that the environment, in interaction with genomics, plays in health, particularly for common diseases such as cancer, cardiovascular and neurodegenerative diseases. This might also be true for the sporadic form of amyotrophic lateral sclerosis (sALS), where both genetic and environmental risk factors are at play with a twin-based estimated heritability of 61%. Thus, with a contribution of ~40%, the contribution of the environment to the risk of sALS may be substantial.

Our understanding of the full impact of environmental stressors on health and disease is however limited by the fragmentation and compartmentalization inherent to approaches so far that examine single or small subsets of environmental stressors at a time. Recognizing this disparity, the EXPOSOME concept was developed reflecting the totality of a person's environmental exposures in space and time, including where we live, the air we breathe, our social interactions and lifestyle such as smoking and exercise, and the extent to which these affect inherent biological functions encoded by our genome. The Exposome is composed of the cumulative measure of environmental influences and associated biological responses throughout the lifespan. It requires the consideration of the kind of exposures (Specific External Exposome), the wider behavioural and social context in which these exposures occur and which can lead to selection of specific external environments (General External Exposome), their impact on the internal environment (Internal Exposome) and their changes over time.

In this presentation I will introduce the concept of the Exposome, discuss methods for quantifying the totality of environmental exposures, and reflect on statistical methods for analyzing Exposome-wide association studies and causal inference using a recently conducted case-control study on sALS.

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C2 UNTANGLING THE ALS X-FILES

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Keywords: ALS untangled, alternative-therapies, reversals

People with ALS (PALS) often consider alternative and off label treatments (AOTs) they find on the Internet. Internet information about AOTs is not always accurate. Harms can occur when PALS try AOTs, even those advertised as "perfectly safe". On the other hand, there are also rare examples of PALS experiencing periods of stability (plateaus) or even dramatic improvements in their function (reversals) that coincide with starting AOTs. Clinicians must decide how they are going to interact with PALS regarding AOTs. Scientists must decide what these individual ALS plateaus and reversals might mean, and how they might be further studied.

Using a case-based format, the first part of this presentation will review the evolution and experiences of ALSUntangled (ALSU), an international program that utilizes shared-decision making to assist PALS considering AOTs. ALSU's unique method will be highlighted, including its Table of Evidence by which each AOT is graded in 5 categories: Mechanism, Pre-Clinical Data, Case Reports, Trials and Risks. ALSU metrics will be presented including its number of Twitter followers, list of requested and completed reviews, and numbers of review downloads. Surprising lessons learned will be shared regarding AOT proponents and the AOTs themselves. While some AOT proponents are clearly "snake oil salesmen", many are "true believers" and have specific, appealing mannerisms that mainstream ALS clinicians might consider including optimism, respect and responsiveness. While some AOTs are bogus, many have plausible mechanisms, and some even have PALS with validated diagnoses and improvements coinciding with their use.

The second part of this talk will focus on the Replication of ALS Reversals (ROAR) Program. This consists of small pilot trials of the exact AOT's associated with the above-described anecdotal improvements. Precedents for serendipitous patient-discovered treatments in other fields will be reviewed. Justification for the first ROAR trial of an AOT called Lunasin will be presented. Patient-centric design features of the ROAR-Lunasin Trial will be highlighted, including wide inclusion criteria, use of

historical controls, substitution of in-person study visits with virtual visits, and availability of results in real time. Preliminary data will be presented including enrollment and retention rates and biomarkers.

The last part of this talk will review the Study of ALS Reversals (StAR) Program. The variable natural history of ALS will be reviewed, including common and uncommon degrees of ALS plateau and reversal. Operational definitions of clinically meaningful "ALS reversal" and "ALS resistance" will be proposed, as well as ways to potentially find more of them. Hypotheses for reversals and resistance and methods for working through these will be reviewed. Preliminary data will be presented, including the finding that ALS resistance appears to be anatomically regional, and is not explained by available demographics, labs or concomitant medications.

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Session 2A RNA Processing and Dysregulation

C3 USING ICLIP TO STUDY THE ASSEMBLY OF PROTEIN-RNA COMPLEXES ASSOCIATED WITH MND

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Keywords: TDP-43, iCLIP, protein-RNA complex

Motor Neuron Disease (MND) is an invariably fatal disease that leads to selective motor neuron (MN) degeneration, muscle wasting and rapid death typically within 25 years of onset. Mutations in several RNA binding proteins (RBPs) cause MND, including TDP43, hnRNPA1, hnRNPA2/B1, FUS and MATRIN3. Disease-causing mutations are most often concentrated within the low-complexity (LC) of these RBPs. We examined how these RBPs assemble into larger protein-RNA complexes assessed with the use of individual-nucleotide resolution UV Cross-Linking and ImmunoPrecipitation (iCLIP), Mass Spectrometry, RNA-Seq and PolyA-Seq. We further focused on the role of the TDP-43 LC domain in protein-protein and protein-RNA interactions, and the functions of these interactions in regulating pre-mRNA processing. Our study indicates that the LC domain serves as a docking platform for protein-protein interactions, which in turn affect the RNA binding properties of TDP-43.

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C4 ALS AND ARTIFICIAL INTELLIGENCE: IBM WATSON SUGGESTS NOVEL RNA BINDING PROTEINS ALTERED IN ALS

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Keywords: RNA binding proteins, artificial intelligence, cerebellum

Background: A number of RNA binding proteins (RBPs) are linked to amyotrophic lateral sclerosis (ALS), with mutations in 11 RBPs causing familial ALS and 5 RBPs associated with ALS pathology with no known genetic mutations. There are over 1450 RBPs in the human genome, and therefore other RBPs may also be linked to ALS.

Objectives: We sought to identify additional RBPs associated with ALS using computational methodologies that analyze prior published information to suggest new RBPs with a connection to ALS. The cognitive capabilities of IBM Watson enable it to extract domain-specific text features from published literature to identify new connections between entities of interest, such as genes, proteins, and diseases. This approach has been successfully applied to gain new insights into oncology, but has not been applied to neuroscience.

Methods: We used IBM Watson to identify RBPs linked to ALS via a training and validation exercise. IBM Watson analyzed published abstracts to learn the text patterns of known RBPs related to ALS, and then applied that learning to a candidate set of proteins ranking them by their text pattern similarity to the known RBP "training set". To test IBM Watson's ability to suggest new RBPs, we first restricted its knowledge to information prior to 2012 and evaluated whether Watson could identify the three RBPs discovered after 2012 (Matrin 3, GLE1, and ARHGEF28). IBM Watson rank ordered 1445 RBPs by semantic similarity to the 8 RBPs known to be involved in ALS prior to 2012.

Results: Matrin 3 was the top candidate in this retrospective analysis, with both ARHGEF28 and GLE1 within the top 10% of all RBPs. Having shown that such approach can successfully identify ALS-associated genes, we applied a training set of all 11 known RBPs mutated in ALS to predict other RBPs linked to ALS. Of the top 50-ranked genes, 5 have already been associated with ALS, with no disease-causing mutations known to date

(RBM45, MTHFSD, SMN2, EWSR1 and hnRNPA3). The top 10 predicted genes were hnRNPU, hnRNPH2, SRSF2, SYNCRIP and CAPRIN1. To validate Watson's predictions, we examined the subcellular distribution of these RBPs in *post-mortem* tissue samples from ALS and control subjects. We identified altered levels and distribution of SYNCRIP in ALS cerebellum. Specifically, SYNCRIP showed increased nuclear immunoreactivity in Purkinje cells in SALS (n=7) compared to controls (n=6), while Purkinje cells from C9orf72-ALS patients exhibited increased cytoplasmic immunostaining for SYNCRIP (n=4). SYNCRIP alterations were also observed in ALS spinal cord. SYNCRIP functions in mRNA maturation and transport, and interacts with TDP-43, FUS, and SMN.

Conclusions: Our approach using IBM Watson to mine scientific literature to find new ALS-linked RBPs has led to exciting findings and may further aid efforts to understand RNA-binding-protein-mediated mechanisms of disease.

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C5 MATRIN-3 REGULATES TDP-43 LEVELS VIA ITS 3'UTR REGION

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Keywords: Matrin-3, RNA, TDP-43

Background: The MATR3 gene encodes for a nuclear matrix DNA/RNA-binding protein known to regulate alternative-splicing and stability of cellular mRNAs. Five different MATR3 mutations have been recently linked to ALS. To date, very little is known of the role that Matrin-3 plays in central nervous system (CNS) function; much less of how these five different mutations lead to motor neuron disease.

Objectives: In this study we aimed to establish a transcriptome-wide map of Matrin-3 binding sites in human brain in an effort to better understand Matrin-3's role in the CNS.

Methods: Cerebellar gray matter from control (i.e. no known pathology) human post-mortem tissue (n=6) were dissected and processed using the well-established individual-nucleotide resolution Cross-Linking and ImmunoPrecipitation (iCLIP) protocol. Six different Matrin-3-specific iCLIP libraries were prepared and sequenced using an Illumina HiSeq machine. A custom, in-house bioinformatics pipeline was used to identify enriched clusters. Target validation was performed in human cultured cell lines using Dual-Luciferase vector systems. Finally, human Matrin-3 was delivered to the adult mouse CNS (FVB mice, n=10) using a novel AAV capsid variant capable of achieving widespread CNS transduction in adult mice following a single IV injection.

Results: Our bioinformatics pipeline identified ~1400 transcripts bound by Matrin-3 in all six human brain samples. As previously reported, Matrin-3 binds primarily to intronic regions (~70%) but also to 5' and 3'UTR regions (~20% combined). Intriguingly, Matrin-3 binding was enriched in the 3'UTR of the TDP-43 transcript. To functionally validate this interaction, we fused the 3'UTR of TDP-43 to the Renilla Luciferase gene in the psicheck-2 vector (Dual-Luciferase System) and co-expressed it with Matrin-3 expressing plasmids. Renilla luciferase activity was reduced to 20% of control levels in the presence of Matrin-3. In contrast, co-expression of the renilla luciferase/TDP-43-3'UTR fusion transcript with an hnRNPA1 expressing plasmid (i.e. negative control) did not result in a loss of renilla luciferase expression when compared to controls. Moreover, deletion of the RNA-recognition domain 2 (RRM2) or of a nuclear localization signal in Matrin-3 abrogates TDP-43 suppression. To further validate this unanticipated interaction we generated novel capsid AAV vector variants encoding for Matrin-3. Delivery of these vectors to adult FVB mice resulted in widespread expression of human Matrin-3 throughout the mouse CNS. Importantly, mouse TDP-43 levels were reduced in mice expressing high levels of AAV-delivered Matrin-3.

Discussion: We have produced the first transcriptome-wide map of Matrin-3 binding sites in human brain. Our analysis shows that Matrin-3 binds the RNA transcripts of a number of ALS-related genes including TARDBP, HNRNPA1 and HNRNPA2B1. Our data also revealed a novel function of Matrin-3: regulation of TDP-43 transcripts. This novel finding has important implications in the molecular pathobiology of motor neuron disease.

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C6 MUSCLEBLIND PROTECTS AGAINST TRANSCRIPTOMIC DYSREGULATION AND NEURODEGENERATION IN FUS MEDIATED ALS

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Keywords: animal models, genetic modifiers, FUS

Amyotrophic lateral sclerosis (ALS) is the most common form of Motor Neuron Disease, and is characterized by the loss of both upper and lower motor neurons. Recently, mutations in genes that encode for RNA-binding proteins have been linked to ALS pathology, suggesting that perturbation of RNA metabolism may be the linked with the disease pathogenesis. Mutations of the gene Fused in Sarcoma (FUS), which codes for the protein FUS, have

been linked to both familial and sporadic forms of ALS. FUS is a DNA/RNA-binding protein that plays critical roles in RNA metabolism including RNA trafficking and alternative splicing.

Using a *Drosophila melanogaster* model for FUS-associated ALS that was developed by our laboratory, we performed an unbiased genetic screen to identify dominant modifiers of ALS-associated neurodegeneration. Unexpectedly, we identified muscleblind (mbl), the *Drosophila* homolog of human muscleblind-like (MBNL) as a strong suppressor of FUS-mediated neurodegeneration *in vivo*. We found that RNAi-mediated knockdown of endogenous *Drosophila* mbl rescues neurodegenerative phenotypes such as retinal degeneration, reduced life span and neuromuscular junction defects caused by ALS-associated mutations in FUS. In order to understand molecular mechanisms of mbl mediated suppression, we performed RNA sequencing using *Drosophila* brains expressing WT or mutant FUS with or without mbl. Our RNA sequencing approach identified several predominantly nuclear genes whose expression is altered by FUS expression, and subsequently returned to almost normal following knockdown of endogenous mbl. Quantitative, reverse transcription, polymerase chain reaction (Q-RT-PCR) confirmed expression changes of identified genes. Taken together, our data suggests an unexpected role of mbl in FUS-mediated neurodegeneration and demonstrates that pathogenic mutations in FUS cause global transcriptomic alterations that can be reversed by depleting endogenous mbl.

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Session 2B Multidisciplinary Management

C7 DEVELOPING AND IMPLEMENTING THE NICE GUIDELINE ON MND

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Keywords: clinical guideline, cost effectiveness

Introduction: The UK National Institute of Health and Care Excellence have issued guidelines on the care of people with motor neurone disease/amyotrophic lateral sclerosis (MND/ALS) looking at riluzole (2001) and non-invasive ventilation (2010). This has now been extended to look at the care of people with MND/ALS using a comprehensive review approach (1).

Methods: Reviews were undertaken looking at information, prognostic factors, organization of care, psychosocial support, end of life care, symptom management, nutrition, communication, respiratory impairment and non-invasive ventilation. A multidisciplinary Guideline Development Group, including patient and carer representatives, made recommendations from the evidence—using GRADE methodology, of randomized controlled trials and cohort studies. In the absence of evidence, recommendations were made by consensus.

Results: Recommendations were made for all areas, particularly:

- The diagnosis should be given by a neurologist with experience in MND care
- Information should be available for patients and families
- A clinic-based multidisciplinary approach should be used, allowing regular assessment of the patient and their needs. A cost effectiveness assessment showed that this approach was cost effective, with a cost of £26,672 per QALY
- Psychological and social aspects should be considered regularly
- Cognitive change should be considered and discussions tailored to the person's capacity, communication and cognitive state
- Symptom management should be assessed regularly—for muscle problems, saliva issues and cough
- Assessment of feeding and nutrition should be undertaken regularly and gastrostomy discussed from an early stage
- Communication needs should be assessed and equipment provided appropriately
- The person with MND should be offered the opportunity to discuss their concerns about the end of life particularly at diagnosis, if there are significant changes in their condition or if interventions are planned
- Equipment should be provided in a timely way, and should be able to be adaptable to cope with deterioration in the patient's abilities
- Regular assessment of respiratory function is essential and non-invasive ventilation should be offered if appropriate, and may be stopped at the patient's request
- Research recommendations were made on cognitive assessment, prognostication, management of sialorrhoea, diet after gastrostomy, provision of communication aids

The challenge is now to encourage all involved in the care of people with MND/ALS to implement these guidelines. This will include community services, neurology services, other supportive medical and allied health professional services, specialist palliative care, social care and the commissioners of these services. The Guidelines aim to provide a clear view of care, supported by the evidence of cost effectiveness.

Conclusion: The comprehensive clinic-based multidisciplinary approach effectively supports MND patients and families, improving both length and quality of life. The challenge is now implementation so that all people with MND/ALS can benefit.

Reference

1. National Institute for Health and Care Excellence. Motor neurone disease: assessment and management. 2016. Available at: www.nice.org.uk/NG42

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C8 LONGITUDINAL ANALYSIS OF PATIENT COMMUNICATION AND TREATMENT PREFERENCES IN AN ALS CLINIC

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Keywords: advance directives, end of life care, goals of care

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: To describe the treatment preferences of patients in an ALS clinic over time as captured by the CTPA.

Methods: The CTPAs were completed by patients at each ALS clinic visit. Completed forms were reviewed for descriptive analysis.

Results: There were 122 patients who completed the CTPA at 27 visits (mean 3.6 visits, 62% men, mean ALSFRS-R score=29, average FVC 59%, and mean ALS-Specific QoL total score of 7.17/10). On their first CTPA, 58% of patients had a financial power of attorney (POA), 51% had a healthcare POA, 35% had an advanced care directive, and 13% had completed a POLST (physician orders for life-sustaining treatment) form. On their last CTPA, each of these increased (65% financial POA; 58% healthcare POA; 46% advanced directive; 28% POLST). On their first CTPA, patients wanted to discuss the following treatments: feeding tube (27%), diaphragm pacing (25%), ventilator support (19%), non-invasive ventilation (25%), and experimental research (39%). On their last CTPA, each of these were lower (16% feeding tube; 11% diaphragm pacing; 11% ventilator; 16% non-invasive ventilation; 28% experimental research). Treatment goals on the first CTPA were comfort (43%), life extension with selected treatments (53%), and extend life at all costs (20%). On the last CTPA, comfort and selected measures remained consistent (44% and 53%, respectively) but life extension at all costs decreased slightly (16%). In a sub-set of patients who completed at least 4 CTPAs (n=48, 52% men), extension of life at all costs at the 4th time point was half of what it was at the 1st (T1: 38% comfort; 40% extension with select treatments; 19% extension at all cost; T4: 40% comfort; 48% select treatments; 10% extension at all cost).

Discussion and conclusions: Although the number of patients whose primary goal is comfort remained stable over time, the number seeking life extension at all costs declined by half in a 9-month period. Experimental research was consistently the most requested treatment discussion despite their goal for care. The CTPA provides a consistent way to assess end of life legal documentation and guide discussions about treatment options that are consistent with the patient's goals for care.

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C9 DETERMINANTS OF THERAPEUTIC DECISION MAKING IN ALS IN GERMANY, SWEDEN AND POLAND

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Keywords: therapeutic, palliative care, QoL

Background: During the course of the disease, ALS patients are forced to make a number of decisions on therapeutic options which may affect Quality of Life (QoL) and prognosis. Preferences may vary considerably between European countries due to legal, religious and cultural backgrounds.

Objective: To define determinants of decision making in a cross sectional comparison between Germany, Sweden and Poland with comparable legal but different cultural and religious backgrounds.

Methods: During 2014–2015 a total of 401 ALS patients were interviewed (Germany (n=265), Sweden (n=71) and Poland (n=65)) on preferences to make use of therapeutic techniques (invasive and non-invasive ventilation (IV and NIV), and percutaneous endoscopic gastrostomy (PEG)) and on hypothetical ideation to terminate these techniques in the course of ALS. Clinical data (ALS-FRS, disease duration), data on wellbeing (quality of life (QoL) with ACSA and SEIQoL, depression with ADF12) religious background (Idler) and personal values (Schwartz Value Scale) were determined. Normal distribution of the data was tested with the Kolmogorov–Smirnov test. For comparison of preferences and wellbeing between countries, analysis of covariance (ANCOVA) with the Scheffé *post-hoc* analysis was computed with clinical data as covariates. To evaluate the relative impact of residency on preferences, multiple regression analyses were conducted with clinical data as covariates. All data were Bonferroni corrected. The significance threshold was set at $p<0.05$. We used SPSS 22.0 for statistical analyses.

Results: Patients' wellbeing was different between countries (in Sweden lower depression scores and in Sweden/Germany higher QoL than Poland all $p<0.05$). Swedish patients were the most autonomous and Polish patients most conservative. Patients had different preferences on therapeutic options in Germany, Sweden and Poland: NIV in Germany and PEG in Sweden were most commonly used in addition to highest preference for usage and ideation to turn off these devices (all $p<0.05$). Polish patients were mostly undecided about the usage of NIV, IV and PEG and were the least likely to show ideation to turn off these devices. Preferences on therapeutic options was only partly explained by medical condition (the more advanced the medical condition the more likely they decided for PEG and NIV with both $p<0.01$). A significant degree of variance was explained by residency ($p<0.05$ for PEG and IV). Preferences for hypothetical ideation to terminate treatments in case of physical decline was determined by residency only ($p<0.001$ for PEG, NIV and IV). Decision status on IV was determined by conservatism and on PEG by autonomy. Wellbeing was a determinant of decision for PEG only. Religiousness was a predictor for decisions for NIV ($p<0.05$) and PEG ($p<0.1$ trend only) and for preferences for hypothetical ideation to terminate treatments.

Discussion: Preferences on therapeutic options are primarily determined by medical condition. However, various other factors such as cultural background have

major impact on decision making in ALS in different European countries.

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C10 COMPARISON OF SURVIVAL OF PEOPLE WITH ALS BY DIAGNOSTIC COHORT

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Keywords: multidisciplinary team, survival, specialist care

Background: Care for people with amyotrophic lateral sclerosis (ALS) has altered over the last 20 years with increased provision of multidisciplinary (MDT) care and specialist ALS clinics. We hypothesized that these changes would improve survival. The King's Motor Nerve Clinic has been a multidisciplinary, specialist, tertiary referral centre since 1995, but has only had a large, experienced team with integrated palliative and respiratory care since 2006. Education and sharing of experience with local care teams has been a priority.

Methods: Patients enrolled in the UK South East ALS population register who had been diagnosed with ALS at King's College Hospital, were studied. Comparison was made between those people diagnosed with definite, probable and possible ALS in the years 1995–1998 and those diagnosed in 2008–2011. Both cohorts were followed up for equivalent periods to avoid bias for very long survivors. Kaplan-Meier survival analysis and Cox multivariate regression were used to analyze the difference between groups.

Results: 546 cases were included in the Kaplan–Meier analysis. There was a significant increase in survival in the 2008–2011 cohort compared to the 1995–1998 cohort (log rank p-value=0.017). 520 cases with complete data were included in the Cox regression. Cohort was a significant predictor variable ($p = 0.017$).

Conclusions: These results suggest that changes in care over the study period have improved ALS survival, and support the hypothesis that integrated specialist clinics, with multidisciplinary input from a wide range of professionals that provide interventional care, improve survival in ALS.

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Session 3A RNA and Neurodegeneration

C11 VULNERABILITY OF MICRO RNAS IN FTD-ALS

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Keywords: microRNA, non coding RNA, dicer

The genetics of the neurodegenerative diseases, amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), are turning scientific attention towards RNA metabolism. MicroRNAs (miRNAs) are endogenous noncoding RNAs that play critical roles in maintaining brain integrity. Accordingly, miRNA dysregulation is involved in several neurodegenerative diseases, including FTD-ALS. miRNAs are susceptible to fail when protein factors that are critical for miRNA biogenesis malfunction. Two ALS-causing proteins, TDP-43 and FUS affect miRNA biogenesis. Furthermore, insufficient activity of other miRNA biogenesis factors, including Dicer, might impair miRNA function in the disease. Specific miRNAs are involved in the regulation of pathways that are essential for neuronal survival or function. Any change in the expression of these specific miRNAs or in their ability to recognize their target sequences will have negative consequences on the motor neuron, exposing new facets of molecular pathogenesis.

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C12 TRANSCELLULAR SPREAD OF MOTOR NEURON DEGENERATION VIA MICRORNAS IN GENETIC MODELS OF ALS

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Keywords: TDP-43, Sarm1, microRNA

Background: The progressive spread of cell death is a hallmark of many age-dependent neurodegenerative diseases including ALS, but the mechanisms remain unclear. Using our *Caenorhabditis elegans* ALS models, we previously discovered that the immune system is inappropriately activated via the protein TIR-1/Sarm1 and contributes to cell death. Inactivating this TIR-1/Sarm1 signalling cascade, either genetically or with drugs alleviates neuronal degeneration caused by mutant human proteins linked to ALS (1). We hypothesize that the expression of mutant proteins linked to ALS leads to an inappropriate paracrine-like activation of the innate immune response via TIR-1/Sarm1 within motor neurons. We believe that an unknown molecule secreted from motor neurons contributes to proximal and distal neurodegeneration.

Objectives: To learn more about disease mechanisms and identify new therapeutic approaches we use the genetic system *Caenorhabditis elegans*, a nematode worm with powerful and rapid methodologies to model ALS. We wish to learn more about the signals involved in the spread of motor neuron degeneration in genetic models of ALS.

Results: To explore the spread of neurodegeneration we constructed a worm model based on the expression of mutant TDP-43 only within GABAergic motor neurons. We observed the degeneration of not only GABAergic motor neurons, but also neighbouring cholinergic motor neurons that do not express TDP-43. This system is suitable for investigating the transcellular transmission of proteotoxic stress and we have identified a micro-RNA essential for the progression of motor neuron degeneration.

Conclusions: We have identified an RNA molecule that is essential for the spread of motor neuron degeneration resulting from the expression of mutant TDP-43 in a discrete neuronal population. The molecular and genetic components of this mechanism are highly conserved and may be new therapeutic targets for ALS drug discovery and development. An update of our findings will be presented.

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C13 CIRCULAR RNA BIOGENESIS IS DEPENDENT ON FUS AND IS IMPAIRED IN AN ALS MODEL SYSTEM

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Keywords: FUS, circRNA, motor neuron

Background: FUS is an RNA-binding protein involved in almost all steps of RNA metabolism, i.e. transcription, splicing, RNA transport and translation (1). Mutations of FUS gene are associated with the onset of Amyotrophic Lateral Sclerosis (ALS) and alterations in RNA metabolism have been described in ALS model systems (2,3).

Objectives: Circular RNAs (CircRNAs) are a new class of RNA that have been recently described. circRNAs are enriched in neuronal tissues (4) and arise from a back-splicing reaction (5). Since FUS has a well-characterized role in splicing regulation, our aims are to understand the role of FUS in circRNAs biogenesis, and to analyse the deregulation of circRNAs in mouse motoneurons, carrying a deletion of FUS gene (FUS KO) or an ALS-associated FUS knock-in mutation (FUS P517L, homologous to human P525L mutation).

Methods: RNAseq of *in vitro* differentiated mouse motoneurons derived from WT, FUS KO and FUS P517L Embryonic Stem (ES) cells was performed. FUS role in circRNA abundance and biogenesis was further studied in differentiated mouse Neuro-2a (N2A) cells.

Results: RNAseq analysis in mouse motoneurons identified about 4000 circRNA species. Interestingly, 136 circRNAs were deregulated in FUS KO samples and 54 circRNAs were deregulated in FUS P517L samples. Notably the deregulation of some of these circRNAs was confirmed in differentiated N2A cells treated with siRNAs against FUS or overexpressing this protein, indicating a correlation between circRNA abundance and FUS levels. Interestingly, the overexpression of two ALS-associated mutant forms of FUS protein, mimic a nuclear loss-of-function of FUS impairing circRNA biogenesis. Cross-Linking and ImmunoPrecipitation (CLIP) experiments revealed that FUS binds the precursor molecules of these circRNAs. Altogether these data suggest a direct role of FUS protein in circRNA biogenesis.

Discussion and conclusions: circRNAs are a new class of RNA molecules with potential pivotal roles in cell homeostasis and differentiation. We identified a class of circRNAs whose expression and biogenesis is dependent on FUS levels and is altered when FUS is mutated. The characterization of the role of FUS in circRNA biogenesis and the identification of some circRNAs deregulated in an ALS-cellular model system open interesting opportunities for the study of this disease.

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C14 LOSS OF TDP-43 CONTRIBUTES TO NON-CODING RNA MEDIATED TOXICITY

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Keywords: TDP-43, RNA, C9orf72

Non-coding RNA species may be toxic via sequestering RNA binding proteins away from their site of action. Indeed, an intronic hexanucleotide repeat expansion in C9orf72, the most common genetic cause of amyotrophic lateral sclerosis and frontotemporal lobar degeneration, forms RNA foci that have been postulated to sequester RNA binding proteins. Importantly, C9orf72 expansion carriers develop TDP-43 pathology, manifest as the loss of nuclear TDP-43 into cytoplasmic aggregates. TDP-43 is a heterogeneous nuclear ribonucleoprotein (hnRNP) that regulates RNA processing. It is unclear how loss of nuclear TDP-43 contributes to neurodegeneration. To better understand the effects of nuclear TDP-43 loss and the C9orf72 mutation, we developed a novel method to fractionate post-mortem human brain to isolate neuronal nuclei with and without TDP-43 for RNA sequencing. Analysis of 1.6 billion uniquely mapped reads from fractionated frontal neocortex of 13 C9orf72 mutation carriers or controls demonstrated widespread transcriptome differences, including 5576 differentially expressed genes (DEGs) due to loss of TDP-43 compared to 323 DEGs linked to the C9orf72 mutation. Global gene expression changes linked to the C9orf72 mutation were highly correlated with reductions in C9orf72 RNA expression. DEG analysis also showed that loss of TDP-43 protein led to altered auto-regulation of the gene encoding TDP-43 (TARDBP). Further analysis showed that loss of TDP-43 was associated with 5337 differentially used genetic elements (DUGEs) with enrichment for non-coding intronic and 3' untranslated RNA segments. Of interest, loss of nuclear TDP-43 was associated with DUGEs that are highly enriched for hnRNP binding sites. Similar changes in non-coding RNA elements were recapitulated upon CRISPR-Cas9 knockout of TARDBP in 293T cells. Moreover, overexpression of non-coding RNA elements altered splicing of TDP-43-dependent CFTR minigene splicing, indicating that these non-coding RNA segments possess biological activity. We propose a model where loss of TDP-43 leads to aberrant non-coding RNA segment processing whereby these non-coding RNA segments contribute to cellular toxicity.

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Session 3B ALS/FTD

C15 ALS/FTD AS A DISORDER OF CONNECTIVITY: WHY ARE COGNITIVE SYMPTOMS AN INTEGRAL PART OF THE ALS SPECTRUM

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Keywords: cognition, behaviour, FTD

For more than a century, our understanding of ALS has been characterized by a "tension between the concept of a selective, purely motor degeneration and a growing realization of the high frequency and importance of cognitive symptoms that can culminate in dementia" (1). The increasingly frequent reports of cognitive and behavioural symptoms in ALS patients seemed to contradict the very idea of a "motor neurone disease" (2). Since the discovery of C9ORF72 in 2011, there has been a remarkable proliferation of studies describing different combinations of frontal-executive, behavioural and language impairment in ALS patients. But in contrast to this wealth of empirical evidence, there have been so far only few attempts to integrate motor, cognitive and behavioural features of ALS into a coherent and unified model of the disease.

A crucial, albeit implicit assumption underlying this tension is that the motor system starts at the primary motor cortex (PMC). Accordingly, ALS can affect the upper and lower motor neurone, but should spare "higher level" cortical areas. In contrast, we propose that the PMC is not the end but the central functional unit of the motor system: the interface between motor cognition (all aspects of cognition which are connected to motor activity) and the execution of movement. If we imagine the motor system as a tree, the upper and lower motor neurons are like its visible trunk and leaves; the motor cognition, in turn, is its invisible but crucially important part, the roots without which the whole system would not be able to function. Once we conceptualize the motor system in this way, the cognitive and behavioural symptoms in ALS become not an unpredictable addition to an otherwise pure clinical picture, but rather an integral part of the dysfunction of the motor system. When examined in more detail, most neuropsychological symptoms in ALS are in some way related to motor cognition: inhibition and activation, judgement and decision making, action knowledge (including verb processing as its linguistic realization) and action planning. Thus, the Frontotemporal Dementia (FTD) encountered in association with ALS can be interpreted as a cognitive disorder of the motor system. The propagation of the disease in ALS/FTD follows these functional connections: "what fires together, dies together" (1). The proposed approach provides an explanation for the co-occurrence of ALS and FTD and builds a bridge between clinical phenotyping on one hand and basic research, from synaptic pathology to neuroimaging of brain connectivity on the other.

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C16 COGNITIVE AND BEHAVIOURAL PROFILES IN FRONTOTEMPORAL DEMENTIA WITH AND WITHOUT AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: frontotemporal dementia, cognitive, behaviour

Background: Approximately 15% of patients with amyotrophic lateral sclerosis (ALS) develop frontotemporal dementia (FTD). The link between the two disorders has led to the notion of a continuum between ALS and FTD. However, it remains an open question whether the cognitive and behavioural impairment in ALS/FTD is identical to that in FTD alone. To date, few direct comparisons have been made between FTD patients with and without ALS.

Objectives: This study aimed to compare patients with ALS/FTD and FTD alone, in order to determine whether the profiles of these conditions can be distinguished on cognitive and behavioural grounds.

Methods and materials: We examined a retrospective cohort of 236 patients with clinical diagnoses of FTD (n=179) or ALS/FTD (n=57) with respect to changes in behaviour, affect, cognition and psychotic symptoms. Features were rated as present or absent based on information recorded from clinical interviews and detailed neuropsychological assessment, carried out at the patients' initial visit.

Results: A number of behavioural and affective changes were reported more frequently in FTD than FTD/ALS: social disinhibition (p<0.001), inertia (p<0.001), loss of sympathy and empathy (p<0.001), and dietary change (p<0.001). Warmth of affect demonstrated in the clinic setting was reported more often in ALS/FTD than FTD (p=0.001). Executive impairments were equally common in both groups. Language impairments were present more often in ALS/FTD than FTD: agrammatism (p<0.033),

impaired sentence comprehension ($p<0.01$) and word finding difficulties in conversation ($p<0.049$). Psychotic features were relatively rare and did not differ significantly between groups.

Discussion and conclusions: Our findings suggest differences between FTD and ALS/FTD. In particular, whilst changes in social behaviour are prominent in FTD alone, there may be a comparatively greater degree of language impairment in ALS/FTD. Prospective exploration of the pattern of differences between these groups will be essential. Identification of a distinct neuropsychological phenotype in ALS/FTD would have clinical implications for early diagnosis, disease management and care planning and theoretical implications for our understanding of the relationship between ALS and FTD.

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C17 COGNITIVE IMPAIRMENT IN MNDS: EXPANDING FROM ALS TO PLS & PMA

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Keywords: cognitive impairment, PLS, PMA

Background: The relationship between ALS and FTD is well known. There is, however, evidence that cognitive impairment not only occurs in ALS but also in the ALS-related motor neuron diseases primary lateral sclerosis (PLS) and progressive muscular atrophy (PMA). Recently, multiple cases of PLS-FTD were identified in the national referral center for motor neuron disease in The Netherlands. The possible relation between PMA and cognitive impairment is especially interesting as PMA is commonly viewed as a motor neuron disease limited to the lower motor neurons.

Objectives: Exploring cognitive impairment in the ALS-related diseases PLS and PMA.

Methods: In the national referral centers for motor neuron disease in The Netherlands cognitive screening has taken place in a research setting in PLS (n=66), PMA (n=89), ALS (n=355) and healthy controls (n=319). The Edinburgh Cognitive and Behavioural Amyotrophic Lateral Sclerosis Screen (ECAS), ALS-FTD-Q and FAB were used as screening instruments.

Results: PLS, PMA and ALS patients performed worse in fluency, executive function and memory compared to healthy controls. Cognitive dysfunction ranged from 10% to 15% in all three motor neuron groups. The ECAS showed a similar cognitive profile in ALS, PLS and PMA patients, except for fluency which was hardly affected in PMA-patients. The behavioural screen of the ECAS was abnormal in 8.3% of the PLS patients and 9.7% of PMA patients. The ALS-FTD-Q showed severe behavioural disturbances in 15% of both PLS and PMA patients, while mild disturbances were found in 17.8% in the PLS group and 11.3% in the PMA group. A FAB score of 12 or lower (the cut-off value) was found in 14% of the PLS patients and 7% of the PMA patients.

Discussion and conclusions: Recently, multiple cases of PLS-FTD were identified in the national referral center for motor neuron disease in the Netherlands. Apart from these clinical findings, cognition was explored in research setting in the motor neuron disorders ALS, PLS and PMA. Interestingly, the percentage of PLS and PMA patients with cognitive dysfunction was in the same range of that of ALS patients (10–15%), emphasizing the importance for cognitive screening as part of routine clinical care in all these three MND patient groups. With a similar cognitive profile, in line with genetic and clinical overlap between the MNDs, the view that PLS is a motor neuron disease exclusively targeting the upper motor neurons and PMA exclusively targeting the lower motor neurons cannot be held. Therefore, our findings are in contrast to the recently revised El Escorial Criteria of 2015, where PLS and PMA are described as restricted phenotypes. We believe that PLS and PMA are multi domain diseases similar to ALS, or are even subtypes of ALS.

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C18 BEYOND THE MOTOR SYSTEM—EXPLORING PSYCHOSIS IN ALS

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Keywords: psychosis, cognition, neuroimaging

Objectives: Psychotic symptoms are being increasingly recognized as a feature of ALS in combination with FTD, and are particularly common in those carrying the C9orf72 expansion. Much less is known of their prevalence in ALS alone, and also of the range of perceptual abnormalities experienced by these patients. This study aimed to (i) determine the rate and severity of psychotic symptoms and perceptual abnormalities in a cohort of patients with ALS with and without cognitive changes, (ii) determine the neural correlates of psychosis in ALS, (iii) examine the underlying cognitive and psychosocial factors involved in psychosis generation in ALS and (iv) examine whether neurodegenerative and psychiatric disorders co-occur within kindreds.

Methods: In total, 100 patients and their carers were enrolled in the study and all underwent a neuropsychiatric interview. In addition each patient completed a battery of questionnaires to determine the presence and absence of

psychosis, mood disorders, perceptual abnormalities and psychosocial factors. All patients underwent neuropsychological assessment and MRI brain scans. Voxel-based morphometric analysis and structural volumetric analysis established patterns of brain atrophy and volume reduction and determined correlations between psychotic symptoms and changes in grey matter. Finally, a familial aggregation study, by means of a validated semi-structured interview, which included 1143 relatives, determined the link between ALS, FTD, C9orf72 and psychiatric disorders.

Results: The rate of psychosis was high across the ALS-FTD continuum (40%) and included ALS patients without cognitive deficits (25%). C9orf72 expansion cases were more likely to exhibit psychotic features than non-carriers ($p < 0.01$). Similarly the rate of perceptual abnormalities was high across the spectrum and included abnormalities of perception in each of the five sense modalities. Neuroimaging linked psychosis with a network of frontal and subcortical structures including the thalamus, striatum and frontal regions ($p<0.01$). Regression models predicted that depression, social isolation and memory deficits account for 45% of the variance in psychosis scores. A markedly increased risk for schizophrenia, autism and suicide was found in relatives of C9orf72 carriers compared to non-carriers (HR—4.9, 2.7 and 2.7, respectively, all $p<0.05$).

Conclusions: This study established that psychosis is not only common in ALS in association with FTD but is also present in ALS without cognitive change and is part of a spectrum of abnormal perceptual experiences. We hypothesize that the generation of psychosis in ALS is due to a complex interaction of brain network abnormalities, cognitive deficits and psychosocial factors. Furthermore, the link between psychosis, psychiatric disease and C9orf72 suggests a common genetic pathway and provides a platform for exploring the underlying biological processes in these conditions.

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Session 4A Protein Misfolding and Aggregation

C19 PROTEINS FOUND IN ALS INCLUSIONS ARE SUPERSATURATED INDICATING PROTEOSTASIS COLLAPSE IN MOTOR NEURONS

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Keywords: proteostasis, protein inclusions, protein aggregate

Background: Amyotrophic lateral sclerosis (ALS) is a heterogeneous and degenerative motor neuron disease that has been linked to numerous genetic mutations in apparently unrelated proteins. These proteins, notably SOD1, TDP-43, and FUS, form a variety of intracellular inclusion bodies that are characteristic of different neuropathological subtypes of the disease. Although many unrelated proteins have been found to co-aggregate with these proteins, it is still unclear which features, if any, distinguish them from other proteins. Here, we show that the 73 proteins reported so far in inclusions of mutant SOD1, TDP-43 and FUS are largely distinct from the native interaction partners of these three ALS-related proteins.

Objectives: To increase our understanding as to why the large set of >70 proteins end up associated with inclusions in ALS.

Methods: We used a data mining approach to identify 73 inclusion-associated proteins in ALS. We estimated the predicted aggregation propensities of each protein using the Zyggregator method, as previously described. Supersaturation is defined as $= C + Z$, where C is the logarithm of the mRNA/protein expression, and Z is the Zyggregator score. We calculated the supersaturation levels of the proteins in ALS aggregates from previously published experimental mRNA and protein concentrations as previously described and compared these scores to the scores of the entire proteome and to the scores of native interactors of SOD1, FUS and TDP-43.

Results: We observe that the particular proteins associated with ALS inclusions are up to ~20 times more supersaturated than the proteome at large, meaning that they have high wild-type cellular concentrations relative to their solubilities. Interestingly, using our original method to calculate supersaturation we find no difference between native interactors of TDP-43, FUS and SOD1 and their co-aggregators, indicating this score alone does not explain their presence in aggregates. However, we then recalculated the supersaturation score using expression data specifically sourced from micro dissected motor neurons or spinal cord tissue data. Here, we find that co-aggregating proteins have an average supersaturation greater than that of the native interaction partners of the main aggregating protein (SOD1, FUS or TDP-43) in motor neurons but not in human tissue at large, indicating that the supersaturation signal in ALS is tissue specific.

Discussion and conclusions: Proteins with a high cellular concentration compared to their predicted solubility (supersaturated) are prone to aggregation when a cellular stress is imposed. One might predict that if protein homeostasis is disturbed, in these specific cells, the supersaturated subset of the proteome would aggregate first. Our results suggest that inclusion bodies in various forms of ALS are produced from a set of proteins that are significantly supersaturated in motor neurons, consistent with a catastrophic protein homeostasis collapse specifically in this cell type.

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C20 TDP-43 AND SOD1: A TOXIC PAS DE DEUX IN ALS

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Background: Misfolded Cu/Zn superoxide dismutase (SOD1) has been detected in all ALS patients, despite SOD1 mutations accounting for only 2% of total cases, while the presence of pathological TDP-43 is a hallmark of all non-SOD1 FALS. We previously reported that SOD1 misfolding is propagated through a tryptophan-32 dependent process (1,2). We also discovered that pathological TDP-43 can trigger the propagated protein misfolding of human wild-type SOD1 in living cells (3,4), but the mechanism has not been elucidated.

Objective: To test the hypothesis that seeding of propagated misfolding of SOD1 is mediated through a homophilic interaction between solvent-exposed tryptophans in TDP-43 and human wild-type SOD1.

Methods: To study the tryptophan-dependence of the intermolecular protein-protein interaction, we synthesized a tryptophan-less cytoplasmic mutant of TDP-43 (TDP-43ΔNLS-Trpless) and assessed its ability to induce misfolding and aggregation of SOD1 in HEK293 cells. We identified misfolded SOD1 using immunofluorescence and quantitative ELISA studies using SOD1 misfolding-specific antibodies. To monitor induced aggregation, we developed a fluorescence-based assay which enables us to monitor SOD1 aggregate formation in living cells. We used small molecules to block this inducible SOD1 misfolding and aggregation.

Results: Immunocytochemical staining, ELISA and flow cytometry reveal that mutant TDP-43 (TDP-43ΔNLS), but not its tryptophan-less version (TDP-43ΔNLS-Trpless), can induce significant misfolding of endogenous wild-type SOD1, as well as aggregation of the fluorescence-based reporter protein. Furthermore, a time-course analysis of aggregate formation reveals that tryptophan-containing mutant TDP-43 induces aggregation 6 times faster than the tryptophan-less variant. Notably, TDP-43ΔNLS cannot trigger misfolding of tryptophan-less SOD1. Using immunofluorescence and flow cytometry, we demonstrated that sub-micromolecular levels of 5-fluorouridine, a small molecule binding tryptophan-32 in SOD1 (5), can efficiently block induced misfolding and aggregation of SOD1.

Conclusion: Pathological TDP-43 can trigger misfolding and aggregation of wild-type SOD1 through protein interaction mediated by their respective tryptophan residues. This interaction can be blocked using small molecules targeting the tryptophan in SOD1, which represents a viable target for ALS therapy. Furthermore, the exposed tryptophan residues in TDP-43 themselves should be identified and explored as potential therapeutic targets. Finally, it is key to recognize that elucidation of the pathogenic role of a simple structural motif in ALS may provide a framework for understanding other neurodegenerative diseases in which propagated protein misfolding is shown to occur (e.g. frontotemporal dementia, Alzheimer's, and Parkinson's diseases).

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C21 CCNF MUTATIONS IN ALS AND FTD LEAD TO DYSFUNCTIONAL PROTEIN HOMEOSTASIS

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Keywords: mutation, CCNF, proteostasis

Background: The only proven causes of ALS are gene mutations that lead to motor neuron death. Much of our understanding of ALS disease mechanisms has stemmed from gene discovery efforts in ALS and ALS/FTD families, highlighting the importance of identifying new causal genes in ALS.

Objectives: We sought to determine the pathogenic role of mutant CCNF through genetic and functional studies, specifically by investigating the functional consequences of the encoded protein in cell models and *post-mortem* tissue.

Methods: We combined linkage analysis and next-generation sequencing technologies in a large ALS/FTD family. Further mutation screening of the causal gene was performed in familial and sporadic ALS and FTD patients. A GFPu reporter assay was used to assess the *in vitro* functional effects on the ubiquitin proteasome system (UPS). We also conducted immunohistochemical staining of *post-mortem* brain stem and spinal motor neurons of an ALS patient with a causal mutation in this gene.

Results: Our previous genome-wide linkage analysis in a large Australian ALS/FTD family identified a novel 7.5 Mb disease locus on chromosome 16p13.3. Subsequent whole-exome sequencing of four affected individuals in the family identified a shared causal missense mutation at this locus, in the CCNF gene, encoding cyclin F (1). Analysis of large international ALS/FTD cohorts identified an additional 14 novel CCNF variants in both familial and sporadic ALS and FTD patients at frequencies ranging from 0.6% to 3.3%. An enrichment of rare protein-altering variants in CCNF was observed among sporadic ALS patients (1.39%) when compared with controls (0.67%), $p=6.58 \times 10-4$. Functional studies indicated that the presence of mutant cyclin F, but neither wild type cyclin F, nor known SNPs in cyclin F, caused the UPS reporter GFPu to aggregate in the cytoplasm, and cyclin F was consistently present in these aggregates. Staining of *post-mortem* brain stem and spinal motor neurons showed mislocalization of TDP-43 from the nucleus to cytoplasm, and ubiquitin- and TDP-43-positive cytoplasmic inclusions in the ALS patient harbouring a CCNF mutation.

Discussion and conclusions: We have reported CCNF mutations in familial and sporadic ALS and FTD cases from diverse geographical locations worldwide. Ongoing functional studies continue to demonstrate that aberrant cyclin F leads to abnormal ubiquitination and UPS dysfunction, implicating common mechanisms, linked to abnormal proteostasis, which underlie neurodegeneration in ALS and FTD.

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C22 TRANSFER OF ALS PROTEIN AGGREGATES BETWEEN MOTOR NEURONS IN THE ZEBRAFISH SPINAL CORD

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Keywords: progression, zebrafish, microglia

Background: The clinical progression of ALS suggests a pathological spread of neurodegeneration through the nervous system (1,2). The mechanisms that are responsible for the spread of neurodegeneration are not known, but are of intense interest because they represent a feasible therapeutic target to halt or delay disease progression. Evidence for a transmissibility or spread of these aggregates is beginning to emerge but limited to *in vitro* studies (3–6).

Objectives: This study investigated the hypothesis that ALS proteins have propagating characteristics, such that insoluble aggregates can transfer between cells and seed aggregation and degeneration in non-affected cells. Our aim was to explore in a living animal model (*in vivo*) the propagation and clearance of ALS aggregates.

Methods: We have expressed ALS aggregates in single motor neurons by injecting zebrafish embryos with DNA constructs of fluorescent wild type and mutant TDP-43 (M337V mutation), or SOD1 (G93A). We applied a UV laser ablation assay to kill the expressing neurons and observe their fate in the spinal cord of zebrafish (6). This novel approach allowed us to visualize the enduring ALS aggregates after ablation and track their movement and progression throughout the spinal cord in real-time.

Results: Our data provides strong evidence that ALS aggregates can survive after the death of a motor neuron. We furthermore demonstrate the intercellular transfer of these aggregates within the spinal cord. Microglial cells get rapidly recruited to the dying neuron and clear the neuronal debris including ALS proteins. We visualized this clearance of neuronal remnants by microglia in real time and characterized the process of microglial engulfment, involving the formation of phagosomes by activated microglia.

Discussion and conclusions: Our novel approach (single neuron ablation and selective expression of ALS aggregates) allowed us to systematically evaluate the fate of released ALS aggregates and their impact on neighbouring neurons and glial cells. We demonstrate *in vivo* the transfer of the persistent ALS aggregates into surrounding cells and reveal their survival after ablation of expressing motor neurons. This real-time visualization of the spread of ALS aggregates in a living organism provides important insights into the pathogenic mechanisms underlying ALS-mediated neurodegeneration and how these aggregates may lead to inflammation and progression of the disease from a focal starting point.

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C23 MODELING NEUROANATOMIC PROPAGATION OF ALS IN THE SPINAL CORD

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Keywords: stochasticity, computational models, anatomical progression

Background: Recent hypotheses of amyotrophic lateral sclerosis (ALS) progression have posited a point-source origin of motor neuron death with neuroanatomic propagation either contiguously to adjacent regions, or along networks via axonal and synaptic connections. Although the molecular mechanism(s) of propagation is(are) unknown, one leading hypothesis is a "prion-like" spread of misfolded and aggregated proteins, including SOD1 and TDP-43.

Aim: To develop a mathematical model representing the cellular and molecular spread of ALS in the human spinal cord.

Methods: Our model is based on the stochastic reaction-diffusion master equation approach using a tetrahedral discretized space to capture the complex geometry of the spinal cord. Domain dimension and shape was obtained by reconstructing human spinal cord from high-resolution magnetic resonance (MR) images and known gross and histological neuroanatomy. Our anatomical model utilizes a stochastic SIR approach (3 states: Susceptible, Infected, Removed) borrowed from spatial epidemiology of infection. In this manner, we modeled neuronal clusters in spinal cord gray matter with a density of 10 cells per volume unit. We then assigned parameters for: (1) probability of a neuron being affected ("infected") on contact by a misfolded protein, (2) amplification rate (generation of additional misfolded proteins by affected

neurons), (3) protein diffusivity and degradation, and (4) rate of the affected neuron's death. We developed this spatial stochastic model in our advanced computational software environment, StochSS (Stochastic Simulation as a Service), which visualizes simulation results with 3D animation videos.

Results: Our model appears to realistically recapitulate the clinical and pathological spread of ALS in human spinal cord. Of note, the speed of longitudinal spread remains constant despite an exponential increase in the amount of misfolded protein. The geometric shape and physical dimensions of spinal cord white and gray matter regions constrain the lateral spread of pathogenic particles and mitigate what would be exponential acceleration. Our results also identify key parameters that require quantification, including the rate of spread of misfolding proteins and the growth rate of protein aggregates.

Conclusion: Future studies will extend our model to: (1) examine spread of misfolded proteins in the cerebral cortex; (2) superimpose spinal and cortical spread; (3) predict the relative contributions of network and contiguous spread to ALS progression; (4) determine whether different initiation locations and distributions lead to disease variability; and (5) examine differential cellular vulnerability in various topographical CNS regions. We will also fit the model parameters to MR imaging data. Finally we anticipate our modeling framework of disease progression will be useful in developing pharmacotherapies (e.g. that inhibit production and/or accelerate degradation/clearance of abnormal proteins) in ALS and possibly other neurodegenerative disorders.

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Session 4B Management of Cognitive and Psychological Change

C24 COGNITION IN ALS: EVOLUTION, INSIGHTS, AND KEY EMERGING TRENDS

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Keywords: cognition, behaviour, caregivers

Cognitive dysfunction is a significant clinical presentation in Amyotrophic Lateral Sclerosis. Interest in, and awareness of, cognitive dysfunction has grown considerably in recent year and our understanding of ALS as a multisystem disease has coincided with genetic and clinical advances linking the continuum of ALS and frontotemporal dementia (FTD). Insights into the heterogeneous cognitive and behavioural profile of ALS patients has not only led to significant clinical and prognostic developments, but also to the recognition of the ever evolving syndrome of cognitive impairment in ALS with recent emerging evidence for social cognitive and language deficits in ALS. Furthermore, the impact of cognitive and behavioural dysfunction on patients and their caregivers is growing, with interest building on the need for caregiver-based interventions. This paper will review the development and rise in our understanding of cognitive and behavioural impairment in ALS and will discuss emerging areas for future development.

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C25 THE RELATIONSHIP BETWEEN COGNITION, BEHAVIOUR, AND DISEASE STAGING IN ALS

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Keywords: cognition, behaviour, disease staging

Background: While cognitive and behavioural symptoms have been clearly demonstrated in patients with ALS, what remains unclear is whether these symptoms decline with disease progression. A recent population-based longitudinal study has suggested a relationship between cognition and functional decline (1). However, previous research has focused on whether cognitive and behavioural symptoms related to functional disease severity or time, rather than disease spread.

Objective: To explore whether cognition and behaviour are related to physical disease spread in ALS.

Methods: 169 incident ALS patients and 81 matched controls were recruited into a prospective multi-centre study. Cognitive and behavioural status was measured using the Edinburgh Cognitive and Behavioural ALS Screen (ECAS), containing ALS-Specific measures of cognition (executive, fluency, and language), and ALS Non-Specific functions (memory and visuospatial). Disease spread was measured using the 4-point King's Staging System.

Results: 25% of patients were in Stage 1, 30% in Stage 2, 12% in Stage 3, and 32% in Stage 4. Analysis of variance of the patient groups and control group reveal significant differences in the ALS-Specific domain of the ECAS ($F(4,226) = 7.219$, $p<0.001$) as well as ECAS total score ($F(3,225) = 7.73$, $p<0.001$). *Post-hoc* analysis corrected for multiple comparisons revealed that for ALS-Specific scores, patients in Stages 3 and 4 significantly differ from controls, while patients in Stages 1, 3, and 4 differ significantly from controls for ECAS Total score. Behavioural impairment, as defined by the presence of two or more ECAS behavioural features, significantly differed across disease stages ($\chi(3) = 10.85$, $p=0.013$). The presence of behavioural impairment in Stage 4 made a significant contribution to the effect with a standardized residual of 2.39.

Discussion and conclusions: While global cognitive impairment was observed in early and late stages of the disease, impairment in ALS-Specific functions were restricted to latter stages. No significant differences were found between disease stages. Conversely, behavioural impairment significantly different between patient groups, with the presence of behavioural impairment in Stage 4 being the most significant contributor. As such, no significant relationship was found between cognition and disease stage, however, behavioural impairment appears to be more prevalent in late-stage patients. Future research should consider disease stage in measuring cognition and behaviour in ALS.

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C26 PREDICTORS OF WELLBEING IN THE COURSE OF ONE YEAR IN ALS

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Keywords: depression, quality of life, predictor

Background: The clinical spectrum of ALS is broad and the same is true for wellbeing: some patients develop depressive symptoms and experience low quality of life, whereas a majority provide evidence for satisfactory wellbeing. The evolution of wellbeing in the clinical context of progressive loss of mobility is scarcely understood.

Objective: To determine predictors of wellbeing in a cross-sectional and longitudinal sample.

Method: In a cross-sectional study, n=276 patients (mean age 60.50 ± 11.85 years) were included within 11.21 ± 15.6 months after diagnosis. Of these, n=54 were measured longitudinally after 12 ± 3.9 months. Clinical data were determined with state of physical function (ALS-FRS), progression of physical function loss, time since onset and time since diagnosis. Depression (ALS depression inventory-12 items, ADI-12), quality of life (QoL: Anamnestic Comparative Self Assessment, ACSA) and wish for hasten death (WHD: Schedule of Attitudes Toward Hastened death, SAHD) were determined as measures of wellbeing. For longitudinal analysis, a pairwise t-test analysis was performed. A linear regression analysis including curve fitting was used for analysis of association of wellbeing (depression, QoL, WHD) and clinical data. The significant threshold was set at $p<0.05$. SPSS 21.0 was used for statistical analyses.

Results: In the longitudinal set, wellbeing was stable within 12 months as there was no significant change in quality of life (p=0.856) or wish for hastened death (p=0.666), whilst depression increased (p=0.01). For the cross-sectional regression analysis, physical function was a significant predictor of wellbeing (QoL R^2=0.048, β=0.219; depression R^2=0.093, β=−0.306; WHD R^2=0.044, β=−0.211 all $p<0.05$, linear curve fitting), in addition to progression rate (QoL R^2=0.043, β=−0.659 and depression R^2=0.089, β=0.625 all $p<0.05$, cubic curve fitting; WHD R^2=0.036, β=0.191 $p<0.05$, linear curve fitting). Time since diagnosis was a significant predictor for quality of life only (R^2=0.060, β=−1.116, $p<0.05$, cubic curve fitting with an increase in QoL after 20 months). Months since symptom onset was no significant predictor (QoL, depression, WHD all $p>0.05$).

Discussion: Within the first two years after being confronted with the fatal diagnosis, ALS patients show an increase of depressive symptoms, whereas other factors of wellbeing (QoL, WHD) were rather stable. Those patients with fast progression rate and low physical function state were at risk for low wellbeing. For those patients with about two years' time after diagnosis, QoL increased again as this subgroup includes only long survivors with slow progression with enough time to adapt to the changed circumstances of ALS.

Conclusion: Within the first two years after diagnosis communication, especially fast progressors are at hazard for low wellbeing. In this crisis-loaded period, these patients need special attention in clinical counseling.

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C27 CAREGIVER BURDEN IN AMYOTROPHIC LATERAL SCLEROSIS: ELUCIDATING THE TRAJECTORY OF PSYCHOLOGICAL DISTRESS IN BURDEN

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Keywords: caregiver burden, longitudinal, cognition and behaviour

Objective: Caregiver burden is a recognized consequence of caring for someone suffering from a neurodegenerative condition. Amyotrophic Lateral Sclerosis (ALS) is rapidly progressive and can impair cognitive function, movement, and behaviour. This rapid disease trajectory separates it from other neurodegenerative conditions. The role of psychological distress in the early stage of the disease, and across the disease trajectory, is unclear.

Methods: Patients with ALS (n=99) and their primary caregivers (n=90) completed serial evaluations investigating the impact of providing care for ALS on caregivers. Anxiety and Depression were evaluated using the Hospital Anxiety and Depression Scale (HADS). The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) was used to measure cognitive function. The ALS Functional Rating Scale (ALSFRS-R) measured disease progression.

Results: Caregivers were categorized as high or low burden, based on statistically derived cut-off scores. In the low-burden group the anxiety score from the HADS (HADS-A) was the only measure predictive of burden (r=0.410, F=3.73, p=0.033). The HADS-D was

predictive of caregiver burden in the high burden group (r=0.501, F=5.87, p=0.006), and these findings were consistent on serial assessment.

Discussion: In a patient cohort with relatively preserved cognitive and behavioural function, anxiety was the best predictor of caregiver burden. Anxiety was apparent in the low burden group of caregivers, prior to clinically significant burden being present.

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Session 5A Therapeutic Strategies

C28 DESIGNING KINASE INHIBITORS TO COMBAT ER STRESS-MEDIATED APOPTOSIS IN A STEM CELL MODEL OF ALS

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Keywords: ER stress, stem cells, neurodegeneration

Background: ALS-causing mutations have been identified in more than a dozen genes; however, emerging theories on the etiology of ALS suggest a role for misfolding, rather than loss-of-function, of the mutant proteins. Insoluble proteinaceous aggregates are a pathological hallmark of motor neurons (MNs) from both familial and sporadic ALS patients (1). Furthermore, there is mounting evidence for the activation of the unfolded protein response (UPR) and endoplasmic reticulum (ER) stress pathways in ALS MNs. These pathways are triggered by misfolded proteins and are initially protective: the UPR halts the synthesis of new proteins and degrades misfolded proteins, and potentiating the UPR increases lifespan in mouse models of ALS (2). If the UPR fails to clear misfolded proteins, it is superseded by ER stress, which triggers apoptosis. Though the precise sequence of events leading from ER stress to apoptosis is still unclear, markers of ER stress have been observed in post-mortem patients, as well as in pre-symptomatic ALS mice (3,4). Together, these findings support the idea ER stress is central to ALS.

Objectives: Our aims are (i) to map the molecular cascades that lead to MN degeneration in the context of ER stress, and (ii) to use these maps to develop new therapeutic compounds for the treatment of ALS.

Methods: Using stem cell-derived MNs, we performed high-throughput screens of small molecules to identify those that selectively kill MNs over other cell types, and found that MNs are particularly sensitive to compounds that trigger ER stress. We then performed secondary screens where we pre-treated MNs with ER stressors, and looked for compounds that rescued their toxic effects.

Results: We identified a family of kinase inhibitors that strongly prevented ER stress-induced apoptosis. Further computational analysis pointed to mixed lineage kinases (MLKs) as their shared putative target. We selected PHB1014, a kinase inhibitor that is highly specific for MLK3 (IC50 = 14 nM) and was particularly potent in our survival assays, to serve as a scaffold for the synthesis of more potent and selective analogs. We synthesized and screened 63 analogs of PHB1014, and found 11 with favorable pharmacokinetic properties that matched or exceeded the capacity of the parental compound to rescue MNs from ER stress.

Discussion and conclusions: We have developed a promising selection of compounds that reverse the toxic effects of ER stress *in vitro*, and are prime candidates for further testing *in vivo* models of ALS.

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C29 MODULATION OF UPR RESPONSE IN IPS CELL-DERIVED MOTOR NEURONS FROM ALS-PATIENTS

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Keywords: unfolded protein response, human induced pluripotent stem cells, phenotypic drug discovery

One pathway strongly activated in ALS is the unfolded protein response (UPR), a signalling network that orchestrates the recovery of homoeostasis or triggers apoptosis depending on the level of protein folding demand in the lumen of the endoplasmic reticulum (ER). Familiar and sporadic ALS patients show accumulation of misfolded proteins and elevated levels of UPR markers. In ALS model mice, studies indicate upregulation of UPR markers early in disease and provide evidence that ER stress correlates with selective vulnerability of specific motor neuron populations. This has generated widespread interest in targeting the UPR response as a therapeutic strategy in ALS. First small molecules targeting different nodes of the UPR are under investigation but highlight the need for molecules with more efficacy, lower risk of adverse events, and a mechanism of action that prevents apoptosis but enhances the buffering capacity of the proteostasis network. We have used motor neurons derived from a panel of well characterized human induced pluripotent stem cell lines, both from familial and sporadic ALS patients, as basic models of disease. In SOD1A4V

motor neurons we show an upregulation of ER stress markers and evidence for electrophysiological differences between mutant motor neurons and isogenic controls. Standardization and upscaling of motor neuron differentiation in 384 well plates allows us to phenotypically screen thousands of small molecule compounds from focused sets ranging from bioannotated known drugs and tools compounds to high chemical diversity and natural product derivatives. Here we present on our phenotypic drug discovery strategy including validation of hits in multiple secondary *in vitro* assays of the UPR pathway, progression towards identification of the underlying molecular targets of selected hits, assessment of selected hits on ALS-specific phenotypes of patient motor neurons, and the testing of selected hits on motor neurons of multiple familiar ALS genotypes. As a result, selected compounds may progress to a lead optimization program and the development of novel drug candidates for the treatment of ALS and further neurodegenerative diseases.

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C30 IDENTIFICATION OF THERAPEUTIC TARGETS FOR CYTOSKELETAL DEFECTS IN ALS

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Keywords: cytoskeleton, neurodegeneration, drug target

Background: The pathogenesis of ALS and the mechanisms that lead to selective motor neuron degeneration are still unknown. This lack of knowledge hinders the development of an effective therapy to prevent or stop progression of the disease. Identification of ALS causative genes has helped to identify potential pathogenic pathways involved in the development of familial and sporadic ALS that can be targeted for therapeutic intervention. We recently identified mutations in two cytoskeletal genes, the actin binding protein profilin 1 (PFN1) and the microtubule subunit α-tubulin 4A (TUBA4A) as causative for familial ALS. These observations suggest that alterations affecting the cytoskeleton architecture, dynamics, and function are important in ALS pathogenesis. Our central hypothesis is that alterations to cytoskeleton structure and dynamics disrupt essential cellular functions, such as synaptic plasticity, vesicle recycling, axonal transport, and neuronal plasticity, which are necessary for the maintenance of motor neurons.

Method: We have developed novel primary neuron cellular models of ALS based on TUBA4A and PFN1 mutations using a custom-built automated longitudinal imaging platform. We apply these cellular models to screen a subset of the druggable genome siRNA library focused on cytoskeletal genes and genes that have direct interactions with known ALS-linked cytoskeletal genes. Screening this RNAi library allows us to identify genes that play a role in the cytoskeleton pathway and act as disease modifiers.

Results and discussion: We have found several modifiers that rescue neurodegeneration caused by mutant PFN1, TUBA4A and TDP43 and could reveal common mechanisms underlying neurodegeneration caused by these mutations. Furthermore, these genes could serve as potential therapeutic targets.

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C31 CHALLENGES IN CNS DRUG DISCOVERY

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Keywords: small molecule drugs, drug discovery, blood-brain barrier

The blood–brain barrier stops many small molecules and most proteins from entering the central nervous system. Fortunately an understanding of the key CNS efflux transporters and how physicochemical properties affect CNS exposure allows the design of small molecule drugs for CNS targets. The discovery of agonists for the 5HT2C receptor will be highlighted to show how physicochemical property optimization and early *in vitro* testing can be used to find CNS active drugs (1). Although historically antibodies and other large proteins have been thought to be completely excluded from the CNS, there is emerging evidence that some therapeutic antibodies can achieve sufficient exposure in the CNS to be effective. Recent examples of the utility of therapeutic antibodies for CNS diseases will be presented.

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Session 5B Pre/Early Symptomatic Disease

C32 DETECTING EARLY CHANGES IN FTD

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Keywords: frontotemporal dementia, cognition, neuroimaging

Frontotemporal dementia (FTD) is a common cause of young onset dementia, approximately equal in frequency to Alzheimer's disease in people below the age of 65. The only known risk factors for FTD are genetic, and around a third of FTD is familial, with three genes accounting for the majority of autosomal dominant FTD: progranulin (GRN), microtubule-associated protein tau (MAPT) and the chromosome 9 open reading frame 72 (C9orf72). Genetic FTD offers a useful model for studying the FTD spectrum, as the underlying pathology is known (inclusions containing the protein TDP-43 in progranulin and C9orf72 mutations and tau inclusions in MAPT mutations) and it allows a window into the earliest changes in the disease process by studying pre-manifest family members who are at risk of developing genetic FTD. However, until recently studies of presymptomatic genetic FTD have been mostly limited to individual case reports or relatively small case series making it difficult to evaluate the pattern of biomarker changes and how they link together. To begin to address this problem, we formed the Genetic FTD Initiative (GENFI), an international multicentre cohort study across Europe and Canada, aiming to facilitate the creation of a common clinical, imaging and biomarkers methodological platform to study a group of individuals affected or at-risk of developing the major genetic forms of FTD. Cognitive, imaging and fluid biomarker studies performed so far have shown that cognitive impairment can be detected around five years prior to symptom onset and structural imaging changes can be seen at least ten years before estimated onset. For the individual genetic subtypes, different gene-specific patterns of neuroanatomical involvement are seen with a medial temporal predominant picture in MAPT mutations, insula and striatal involvement in GRN mutations and thalamus and cerebellar atrophy in C9orf72 mutations. Changes in structural and functional connectivity networks can be seen earlier than grey matter atrophy. Few fluid biomarkers have been investigated but both serum and CSF neurofilament light chain concentrations appear to correlate with disease intensity in genetic FTD. GENFI has now recruited over 500 individuals and will continue in the current phase until 2020. The key outcomes from the study will be: robust markers of disease onset; markers indicative of optimal time to start disease-modifying therapy; robust markers of disease progression that can be used as outcome measures in trials, and creation of a large cohort of presymptomatic at-risk genetic FTD participants available to participate in clinical trials.

C33 QUANTITATIVE MOTOR TESTING: BIOMARKER OF PRE-SYMPTOMATIC ALS?

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Keywords: pre-symptomatic, quantitative motor, biomarker

Background: It is widely believed that there is a pre-symptomatic/pre-manifest phase in ALS, in which the disease process unfolds prior to the emergence of symptoms. Since pre-symptomatic disease is, by definition, characterized by the absence of overt clinical manifestations, measures that are sensitive to early motor neuron loss are essential to exploring the onset and progression of disease in the pre-symptomatic population. Quantitative-motor (Q-Motor) paradigms, originally developed for Huntington's disease (HD), have been shown to detect and longitudinally track motor dysfunction in pre-manifest HD gene carriers up to two decades prior to HD onset. They are objective and have been standardized across centers, exhibiting no placebo effects in HD clinical trials. Consequently Q-Motor measures are currently in use as outcomes in several HD trials.

Objectives: To investigate the utility of Q-Motor measures in detecting pre-symptomatic motor dysfunction in unaffected individuals at-genetic-risk for developing ALS (participants in the Pre-Symptomatic Familial ALS (PrefALS) study), including the small subset who phenoconverted (progressed to clinically manifest disease) during longitudinal follow-up.

Methods: Q-Motor tasks were performed longitudinally in at-risk individuals, ALS patients, and healthy controls. The clinical burden of motor pathology in ALS patients was graded based on the presence of atrophy, changes in muscle tone and reflexes, and the degree of weakness. Specific Q-Motor tests included grasping, tapping and force matching tasks using a pre-calibrated force-transducer (Mini-40, ATI) assessing the coordination, speed and variability of voluntary movements. During the lifting tasks an electromagnetic 3D sensor (Fastrack, Polhemus) recorded the object's position in the x–y–z (position-index) and roll–pitch–yaw axes (orientation-index).

Results: Study population includes n=45 controls (64 person-visits), n=78 at-risk individuals (n=52 SOD1+, n=20 C9ORF72+, n=6 other genetic mutation; 152 person-visits), and n=38 affected individuals (63 person-visits). Preliminary analysis of lifting task data has been completed and is presented here. Position- and orientation-indices (reflecting motor performance in holding the

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object steady in space) showed a gradient of worsening performance across the spectrum of controls, at-risk individuals, and ALS patients with increasing clinical burden of motor pathology. In longitudinal analysis, these indices improved slightly over time (suggesting a possible practice effect) in control and at-risk individuals, but declined over time in the ALS population. In three at-risk individuals who developed ALS (none with arm onset) and in whom Q-Motor data were available before and after symptom onset, motor performance declined prior to phenoconversion. Moreover, their motor regions unaffected at the time of phenoconversion also displayed impaired motor performance in Q-Motor measures.

Discussion: While data analyses from other Q-Motor tasks are still ongoing, these preliminary observations already suggest that Q-Motor testing may reveal subtle motor dysfunction in the clinically (as yet) unaffected motor region(s) of patients with early ALS—and perhaps even in at-risk individuals before phenoconversion.

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C34 CORTICAL DYSFUNCTION IS A GLOBAL PHENOMENON IN ALS

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Keywords: global, cortical dysfunction,

Background: Amyotrophic lateral sclerosis (ALS) is characterized by upper and lower motor neuron dysfunction, with disease progression evolving rapidly across body regions. Although cortical hyperexcitability has been identified as an early and intrinsic feature of ALS, linked to neurodegeneration, the issue of whether cortical dysfunction is a focal or global cortical phenomenon remains unresolved.

Objectives: The present study attempted to assess the focal or global nature of cortical dysfunction which could be of pathogenic significance, potentially accounting for the patterns of disease spread, as well as providing guidance for novel therapeutic significance.

Methods: Assessment of cortical function was undertaken in 50 ALS patients (32 males, 18 females, mean age 60 years), diagnosed according to the Awaji Criteria, utilizing the threshold tracking transcranial magnetic stimulation (TMS) technique. Motor evoked potential responses were recoded over the abductor pollicis brevis (APB) muscle bilaterally and all patients were longitudinally monitored.

Results: Cortical hyperexcitability was evident in both motor cortical hemispheres, and evidenced by a significant reduction of SICI (SICI CONTROL $10.4 \pm 0.7\%$; SICI RAPB $4 \pm 1.3\%$, $p<0.001$; SICI LAPB $4.9 \pm 1.2\%$, $p<0.001$). The reduction of SICI was accompanied by an increase in MEP amplitude (MEP CONTROL $24.7 \pm 1.7\%$; MEP RAPB $40.9 \pm 4.5\%$, $p<0.005$; MEP LAPB 31.8 ± 3.2, $p<0.05$) and reduction of cortical silent period duration (CSP) (CSP CONTROL 208.2 ± 3.0 ms; CSP RAPB 180.0 ± 5.7 ms, $p<0.001$; CSP LAPB 185.8 ± 5.5 ms, $p<0.005$). The degree of cortical hyperexcitability was comparable over both hemispheres in limb-onset ALS patients, irrespective of site of disease onset or extent of lower motor neuron dysfunction ($p=0.4$). In bulbar-onset ALS patients, cortical hyperexcitability was more prominent when recoded over the dominant limb (SICI DOMINANT $3.0 \pm 2.6\%$; SICI NON DOMINANT 7.0 ± 1.9; $p<0.05$), despite a comparable degree of lower motor neuron dysfunction between the sides.

Discussion: Cortical hyperexcitability appears to be a global cortical phenomenon, occurring at early stages in the disease process. Although the degree of cortical hyperexcitability was comparable between the hemispheres in limb onset ALS, it appeared to be more prominent in the dominant hemisphere in bulbar onset ALS, suggesting heterogeneity of the cortical dysfunction. Strategies aimed at modulating global motor cortical function in both motor cortices are likely to prove therapeutically effective.

Conclusion: Cortical hyperexcitability appears to be a global and early feature in ALS, potentially underlying the clinical pattern of spread in ALS.

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C35 BLOOD BIOMARKERS OF CARBOHYDRATE, LIPID AND APOLIPOPROTEIN METABOLISM AND RISK OF AMYOTROPHIC LATERAL SCLEROSIS: A MORE THAN 20 YEAR FOLLOW-UP OF THE SWEDISH AMORIS COHORT

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Keywords: metabolism, lipids, blood biomarkers

Background: Impaired carbohydrate and lipid metabolism have been suggested to precede motor symptoms in animal models of ALS. Corresponding evidence in humans is lacking.

Objectives: The aim of this prospective cohort study of more than 20 years of follow-up was to assess the associations of several carbohydrate and lipid biomarkers with the future risk of ALS, and to specifically examine for the first time the associations of apolipoprotein B (apoB) and apolipoprotein A-I (apoA-I) with the future risk of ALS.

Methods: In the Apolipoprotein-related MOrtality RISk (AMORIS) study, we enrolled 636,407 men and women during 1985–1996 in Stockholm, Sweden with measurements of serum glucose, fructosamine, total cholesterol, LDL-C, HDL-C, triglycerides, apoB and apoA-I. The cohort was followed until the end of 2011 and incident cases of ALS were identified from the Swedish Patient Register. We used Cox models and mixed-effects models to first estimate hazard ratios (HRs) for the associations between these blood biomarkers and ALS incidence, and secondly to assess the temporal changes of these biomarkers during the 20 years before ALS diagnosis.

Results: One unit increase of LDL-C (HR=1.14, 95% CI=1.02–1.27), apoB (HR=1.68, 95% CI=1.17–2.42) and apoB/apoA-I ratio (HR=1.90, 95% CI=1.29–2.78) was associated with a higher incidence of ALS. High glucose level ($\geq$6.11 mmol/L) was associated with a lower incidence (HR=0.62, 95% CI=0.42–0.93), whereas high LDL-C/HDL-C ($\geq$3.50; HR=1.50, 95% CI 1.15–1.96) and high apoB/apoA-I ($\geq$0.90 for men and $\geq$0.8 for women; HR=1.41, 95% CI 1.04–1.90) ratios were associated with a higher incidence of ALS. During the 20 years before diagnosis, ALS patients had lower levels of glucose, whereas higher levels of LDL-C, the LDL-C/HDL-C ratio, apoB and the apoB/apoA-I ratio compared to individuals without ALS. During the 10 years before diagnosis, ALS patients tended to have increasing levels of LDL-C, HDL-C, apoB and apoA-I, whereas quickly decreasing levels of LDL-C/HDL-C and apoB/apoA-I ratios.

Discussion and conclusions: The temporal patterns noted during the 20 years before diagnosis suggest that altered energy metabolism might be both a preceding and succeeding event to neuro-degeneration in ALS. The importance of balances between LDL-C and HDL-C as well as between apoB and apoA-I on ALS development needs to be further studied.

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Session 5C Autonomy and Quality of Life: The Patient–Carer Dyad

C36 PHYSICAL AND PSYCHOLOGICAL INFLUENCES UPON QUALITY OF LIFE IN MOTOR NEURONE DISEASE/ALS

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Keywords: quality of life, self-esteem, self efficacy

Background: Both psychological and physical factors may be expected to influence quality of life (QoL) in MND/ALS; for example, self-esteem has been demonstrated to predict well-being in the condition (1). Self efficacy, the confidence in one's ability to manage the condition, has been shown to mediate pain (2).

Objectives: To assess the associations between self-esteem, self efficacy, physical aspects and QoL of those with MND/ALS in a large population-based sample.

Methods: The Rosenberg Self-Esteem Scale (RSES), Generalised Perceived Self Efficacy Scale (GPSE), ALS Functional Rating Scale-revised (ALSFRS-R), and WHO QoL-Bref were collected as part of the TONiC study, a multicentre, UK study of factors affecting QoL in MND. Both the RSES and GPSE scores were grouped into tertiles representing low-medium-high levels of the respective constructs. The single item of the WHO QoL-Bref, which summarized the overall perception of QoL, was grouped into either (1) very poor/poor/neutral, or (2) good/very good, and used as a dependent variable in a conditional logistic regression to predict good QoL. The Bulbar, Motor and Respiratory scores of the ALSFRS-R were also included in the model, along with demographic and clinical variables.

Results: 465 records were available for analysis by March 2016. Mean age was 64.9 years, median disease duration 11 months, 60.6% were male. There was a strong association between self-esteem and self efficacy (χ^2 = 120.9: p<0.001). Over a third (36.9%) displayed high levels of both self efficacy and self-esteem, whereas 10% displayed low levels of both constructs. A conditional forward Regression (Hosmer and Lemeshow 0.286; Nagelkerke R^2 = 0.3480), correctly allocating to a good/very good QoL in 74.4% of cases, showed that self-esteem, self efficacy, and the Motor subscale of the ALSFRS-R were all significant predictors. A high self-efficacy increased the odds of good QoL (compared with low) by 20.5 (95% CI: 2.6–161.8), and high self-esteem increased the odds of good QoL (compared with low) by 17.6 (95% CI: 2.1–149.2). Each point on the ALSFRS-R Motor subscale (where a high score is good) increased the odds of a good QoL by 1.067 (95% CI: 1.02–1.11). Age, gender, time since diagnosis and site of onset, together with Respiratory and Bulbar scores from the ALSFRS-R, did not reach significance in the model.

Conclusion: Both self efficacy and self-esteem are shown to independently contribute to a good QoL in those with MND/ALS, adjusted for each other and the level of mobility. This suggests they could be targeted for intervention to support continuing good QoL.

Acknowledgements: We thank the participants, without whom this study would not be possible, and also MND Association UK and NIHR CRN for support.

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C37 SUBJECTIVE PERCEPTION OF HEALTH IN ALS: A MOVING TARGET?

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Keywords: EQ-5D, ALSFRS-R, depression

Background: There is no consensus on the optimal instrument to measure quality of life (QoL) in ALS. Distinctions may be made between individual quality of life (QoL), subjective health-related QoL (HR-QoL), and objective (i.e. societal perception) HR-QoL in single or multiple domains.

Objective: To examine determinants and course of subjective perception of health in a large cohort of ALS patients.

Methods: Subjective perception of health on a visual analog scale (EQoL-VAS, marked from 0 to 100, a part of the Euro-QoL EQ-5D) and other self-report measures were obtained during routine clinical care by employing a software system (Knowledge Program) on tablet devices. Uni- and multi-variable linear models, mixed effect models and structural equation models were used for analysis.

Results: Between 2010 and 2015, 578 ALS patients recorded at least one EQoL-VAS. Median initial EQoL-VAS was 60 (inter-quartile range 47–77). EQoL-VAS diminished with ALSFRS-R, although the slope of decline decreased at lower ALSFRS-R. In bulbar-onset disease, EQoL-VAS was about 10 points higher, adjusting for ALSFRS-R. In multivariable models, decreasing gross motor and respiratory subscores of ALSFRS-R, and increasing depression (measured by PHQ-9) had large,

highly significant and monotonous negative effects on EQoL-VAS. The effect of decreasing fine motor subscore was non-linear, whereas the effect of decreasing bulbar subscore was paradoxical, causing an increase in EQoL-VAS after adjusting for other variables. EQoL-VAS declined at an average rate of 0.6 points a month on follow-up. The EQ-5D index, a unidimensional measure of objective HR-QoL, ignored and underestimated contributions of the respiratory subscore and depression on QoL, respectively.

Conclusion: Subjective perception of health in ALS measures physical and psychological impairment, and declines with progression of disease. Among physical factors, the paradoxical effect of bulbar impairment on improving subjective perception of health requires further study.

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C38 ARE CAREGIVERS ABLE TO CORRECTLY PREDICT ALS PATIENTS' WISH FOR HASTENED DEATH AND THEIR WELL-BEING?

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Keywords: caregiver, SAHD, quality of life

Background: ALS patients and their caregivers are confronted with existential decisions concerning life-prolonging and -shortening measures during the course of the disease. Ideally, these decisions are the results of interactions between patients, physicians and caregivers. In case of inability to decide (e.g. due to progressive paralysis) physicians and caregivers are asked to decide on behalf of the patient in the sense of surrogate decision making. Important factors of patients' preferences are their wish for hastened death and their well-being, which need to be taken into account by surrogates to best meet the patients' will in decision making.

Objective: To investigate the extent of caregivers ability to correctly predict ALS patients' wish for hastened death and their overall well-being.

Methods: 61 patients with ALS and their caregivers (N = 35 spouses/life partners, N = 11 children, N = 2 good friends, N = 13 other relatives) were recruited from four different German ALS clinics. Patients and caregivers completed the German versions of the Schedule of Attitudes toward Hastened Death (SAHD; patients only), the ALS Depression Inventory (ADI-12) and the Anamnestic Comparative Self Assessment (ACSA). Additionally caregivers were asked to complete these questionnaires from the patient's perspective.

Results: ALS patients' desire for hastened death, rated on a scale from 0 = non-existent to 20 = very strong, was rather weak (mean SAHD-score: 5.57; standard deviation: 3.85; range: 1–20). Caregivers rated patients' desire for hastened death in a similar range on average (SAHD-score: 5.84; standard deviation: 3.91; range 1–19). However, for pairwise comparison caregivers' rating was completely independent of patients' and did not correlate with their actual desire for hastened death (R^2 = 0.04; p = 0.175). The average deviation was at 3.29 and we observed severe over- and underestimations, i.e. patients SAHD-score and their caregivers prediction of it differed between +12 and −12. Quality of life and depressiveness were not significantly different between patients and their caregivers. Yet, caregivers estimated patients' depressiveness as significantly higher ($p < 0.001$) and their quality of life as significantly lower ($p < 0.01$) than actually reported.

Discussion and conclusions: Caregivers' estimation of ALS patients' attitude toward hastened death bears no relation to their actual desire. There is a comparably large discrepancy between these two measures and patients' wish for hastened death has been strongly over- and underestimated by their caregivers. Also, caregivers rated their diseased relatives' well-being significantly worse than patients did themselves. This finding has considerable consequences in the context of surrogate decision making, as a good communication between ALS patients and their caregivers, especially about life-prolonging and -shortening measures is vital to ensure adequate realization of patient's will concerning these decisions.

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C39 JOURNEY TO ALS DIAGNOSIS – CAREGIVER PERSPECTIVES

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Keywords: caregiver, symptoms, diagnosis

Background: A considerable amount of care for people with ALS is provided in the community by family members. The diagnosis of a terminal illness not only affects the life of the patients but also can have a dramatic impact on the lives of the family members, some of whom will become caregivers, and can impact on their ability to prepare and transition into their new role.

Objectives: To explore the journey from first symptoms to diagnosis from the perspective of informal caregivers of people with ALS in Ireland.

Methods: Caregiver participants were recruited as part of a longitudinal study of people with ALS, and their primary informal caregiver attending a multidisciplinary clinic in Dublin. In a semi-structured interview, caregivers were

asked about their experiences from the time of symptom onset to ALS diagnosis. Thematic analysis is used to identify, analyse and report themes from caregiver responses.

Results: This analysis is based on data from 76 caregivers. This group is predominantly female (70%), a majority are spouses/partners of ALS patients (71%) and 21% are adult children. The average age is 55 years. Themes developed include: (1) noticing problems/symptoms e.g. difficulties with walking and swallowing (2) responding to these problems e.g. avoidance, denial, fear, worry, frustration, seeking information and help (3) interaction with health care services and engagement with health care professionals.

Discussion and conclusions: Caregivers recalled early problems which were often attributed to other conditions, and retrospectively recognized an evolving pattern of symptoms. Realizing something was wrong provoked fear, worry, and frustration, while others denied or avoided confronting a deteriorating situation. Interaction with the health services involved getting referrals and relevant interventions, and communication with health care professionals which was recalled as less than optimal for many. Family caregivers are key figures in care provision, and provide emotional and physical support for patients and often play a central role in clinical decision-making. The time from first symptom to diagnosis with ALS/MND is a crucial time for future caregivers, and their experiences could impact on their ability to cope with their future role in a progressive and rapidly changing condition with increasing demands on time and personal resources.

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Session 6A Cell Biology and Pathology

C40 ANGIOGENIN, tiRNA AND VASCULAR INTEGRITY IN HEALTH AND DISEASE

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Keywords: angiogenesis, non-coding RNA, paracrine signalling

Loss-of-function mutations in the gene encoding for angiogenin (ANG) occur in familial and apparently sporadic ALS patients, progressive muscular atrophy, and recently, Parkinson's disease. ANG encodes a 14-kDa angiogenic ribonuclease that is a hypoxia-inducible factor and acts as a permissive factor for other angiogenic factors to induce angiogenesis. While angiogenin was originally identified as a potent inducer of neovascularization, previous work focused on the actions of angiogenin in motoneurons in which it is highly expressed. Angiogenin protected cultured motoneurons against ALS-associated, stress-induced cell death by promoting and sustaining cell survival signaling through PI-3-Kinase/Akt kinases, and delivery of recombinant human angiogenin protein increased life-span and improved motor function in SOD1G93A mice. Recent work, however, suggested angiogenin works in paracrine to control the translational output of neighbouring cells, including astrocytes, microglia, and endothelial cells. After it is stress-induced and secreted from motoneurons, angiogenin is subsequently taken up via clathrin-mediated endocytosis into non-neuronal cells. Uptake of angiogenin into astrocytes and endothelial cells induces a unique pattern of RNA cleavage, and leads to the formation of specific tiRNAs, a novel class of non-coding RNAs generated from transfer RNAs. In the nucleus of endothelial cells, angiogenin stimulates rRNA transcription required for their proliferation. In line with a pleiotropic activity of angiogenin, systemic delivery of angiogenin is sufficient to reverse the decrease in microvascular density in the lumbar spinal cord of SOD1G93A mice and to delay the decrease in motor function. These studies highlight the importance of maintaining vascular integrity during disease progression, and suggest that germline mutations in ALS-associated genes may also impact on vascular function.

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C41 APICAL DENDRITE DEGENERATION, A NEW CELLULAR PATHOLOGY IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: Betz cells, upper motor neurons, ALS

Background: Upper motor neurons (corticospinal motor neurons (CSMN) in mice and Betz cells in humans) are important for the initiation and modulation of voluntary movement, and their degeneration is a hallmark of amyotrophic lateral sclerosis (ALS) and ALS with frontotemporal dementia (FTD-ALS). Developing evidence reveals that their cellular degeneration is an early event in the disease, albeit detailed mechanisms remain mostly unknown. We previously found profound apical dendrite degeneration in CSMN that become diseased due to different underlying causes, such as mSOD1, absence of Alsin function, and problems with UPS. This suggested that CSMN connectivity was affected early in the disease due to cellular defects, especially at the apical dendrite.

Objectives: ALS mouse models display CSMN abnormalities manifested by spine loss and apical dendrite degeneration early in disease, however health and integrity of apical dendrites of Betz cells in ALS patients have not been assessed previously. Here, our goal is to investigate whether findings from diseased CSMN in well-characterized mouse models are recapitulated in Betz cells of ALS patients, and whether apical dendrites of Betz cells and other neurons in the motor cortex are affected in the disease.

Method: In order to assess neuronal integrity of Betz cells, primary motor cortices were isolated from post-mortem normal control subjects, familial ALS patients, sporadic ALS patients, FTD-ALS, and AD (Alzheimer's disease) patients, and subjected to Map2 immunohistochemistry. Detailed cellular analyses were focused on apical dendrites and soma of large pyramidal neurons in layer V of the motor cortex.

Results: We find that Betz cells of both familial and sporadic ALS as well as FTD-ALS patients display profound degeneration, especially at the site of the apical dendrite where numerous apical dendrites were filled with vacuoles. Even though vulnerable neurons in the hippocampus of AD patients displayed major cellular defects in

soma, Betz cells of AD patients and healthy controls retained cellular integrity in the motor cortex.

Discussion: These findings demonstrate that apical dendrite degeneration observed in diseased CSMN of ALS mouse models is recapitulated in Betz cells of ALS patients. The profound cytoarchitectural defects, observed especially within apical dendrites support the hypothesis that the cortical connectivity defects of Betz cells contribute early to disease pathology in ALS. Thus, improving the health and connectivity of Betz cells early in the disease would offer a novel opportunity for therapeutic interventions.

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C42 DYSHOMEOSTASIS OF COPPER PROTEINS IS A COMMON FEATURE OF SPORADIC HUMAN MND AND TRANSGENIC MOUSE MODELS: OUTCOMES FROM A NOVEL METALLOPROTEOMIC ANALYSIS

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Keywords: copper, human sporadic MND, metalloproteomics

Background: Copper is an essential element. It is bound to a wide range of proteins within every cell type and confers catalytic activity to many diverse enzymes, including copper/zinc-superoxide dismutase (SOD1), the first established cause of familial MND. Copper dyshomeostasis is implicated in various forms of MND and therapeutically modulating copper bioavailability in transgenic MND model mice protects motor neurons, improves locomotive function and extends survival (1,2). Thus, there is increasing evidence that copper and copper-proteins are involved in MND pathogenesis and that they may represent new opportunity for therapeutic intervention. However, a comprehensive understanding of the extent of copper protein dyshomeostasis in MND is yet to be established.

Objective: To determine if dyshomeostasis of copper proteins occurs in human MND-affected spinal cord.

Methods: We applied metalloproteomic techniques to assess changes to copper proteins in spinal cord tissue from mutant SOD1 mice as well as post mortem tissue from human sporadic cases of MND. The methodology provides broad coverage of soluble proteins in spinal cord extracts and quantitative information on the amount of copper bound to these proteins.

Results: The copper-protein profile reveals considerable differences between control tissue and MND. Comparable changes were observed in spinal cord tissue from the mutant SOD1 mice. The strongest link between sporadic human MND and the transgenic models was in a novel copper-protein and not SOD1 as hypothesized. We show that for many metals (zinc, iron, manganese, etc.) their associated metalloprotein profiles are unchanged in human MND-affected spinal cord tissue, increasing the validity of the changes in the copper-proteins.

Discussion and conclusions: Our application of a novel metalloproteomic approach has shown that changes to copper-proteins are a feature common to human sporadic MND and animal models of familial MND. These outcomes indicate that therapies that restore the copper status of these proteins may be a viable treatment option for both familial and sporadic forms of the disease.

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C43 SRSF1-DEPENDENT NUCLEAR EXPORT INHIBITION OF C9ORF72 REPEAT TRANSCRIPTS PREVENTS NEURONAL DEATH AND ASSOCIATED MOTOR DEFICITS

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Keywords: C9ORF72, RNA nuclear export, therapeutic strategy

The most commonly identified cause of ALS and FTD involves autosomal-dominant repeat expansions of the GGGGCC sequence in the first intron of the C9ORF72 gene (1,2). Repeat transcript expression and repeat-associated non-ATG (RAN) translation into dipeptide-repeat proteins (DPRs) trigger complex mechanisms of neurotoxicity. Intron-containing repeat transcripts are notably able to escape protective nuclear retention and become translated into toxic DPRs in the cytoplasm. Nucleocytoplasmic transport defects were recently highlighted in Drosophila, yeast and human neuronal models of C9ORF72-ALS (3–6). However, the mechanism(s) driving the nuclear export of C9ORF72 repeat transcripts remain to be elucidated. We reported that the non-essential nuclear export adaptor proteins SRSF1 (serine/arginine-rich splicing factor 1) and ALYREF (Aly/REF export factor) directly bind hexanucleotide repeat RNA (7). They can further be sequestered by RNA foci (7,8).

In this study, we hypothesized that excessive binding of export adaptor(s) might force interactions with the nuclear export machinery and override the mechanisms of nuclear retention. Depletion of factors involved in inappropriately licensing the nuclear export of repeat transcripts might therefore confer neuroprotection. Here we show that partial depletion of SRSF1 prevents neurodegeneration *in vivo* and rescues locomotor deficits in an established Drosophila model of C9ORF72-ALS expressing uninterrupted repeat transcripts and DPRs (9). This intervention also prevents the death of motor neurons in co-culture with astrocytes derived from C9ORF72-ALS patients. Moreover, we show that depleting SRSF1 or blocking its interaction with the NXF1 export receptor inhibits the nuclear export of C9ORF72 repeat transcripts and the subsequent RAN translation of sense and antisense DPRs to rescue neurotoxicity. This is the first study to identify the molecular mechanisms driving the nuclear export of repeat transcripts. It reveals that the hexanucleotide repeat expansions are sufficient to trigger their own nuclear export via an interaction between the nuclear export adaptor SRSF1 and the export receptor NXF1. In addition, we show for the first time that manipulating the nuclear export of repeat transcripts provides a promising neuroprotective strategy for C9ORF72-ALS.

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C44 THE DNA DAMAGE RESPONSE (DDR) IS INDUCED BY THE C9ORF72 REPEAT EXPANSION IN ALS

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Keywords: C9ORF72, DNA damage, DRP's

Background: Hexanucleotide (GGGGCC) repeat expansions in a non-coding region of C9ORF72 are the major cause of familial ALS (~40%) and FTD (~20%) worldwide. The C9ORF72 repeat expansion undergoes repeat-associated non-ATG (RAN) translation on both sense and antisense strands, resulting in expression of five dipeptide repeat proteins (DRPs), including poly(GR) and poly(PR). Whilst it remains unclear how mutations in C9ORF72 lead to neurodegeneration in ALS and FTD, recently, nucleolar stress and R loop formation were implicated as pathogenic mechanisms. These events can occur during normal cellular processes, but they damage DNA and hence are a serious threat to genome integrity. DNA damage occurs in many forms, but double-stranded breaks (DSBs) are the most cytotoxic lesions, and the cell activates the DNA damage response (DDR) with the aim of repairing this damage.

Results: In lumbar spinal cord motor neurons from C9ORF72-positive ALS patients, using immunohistochemistry and immuno-blotting, we demonstrated significant up-regulation of markers of the DDR compared to control subjects: phosphorylated histone 2AX (-H2AX) (35% increase; $p<0.001$), phosphorylated ataxia telangiectasia mutated (p-ATM) (42.32% increase; $p<0.05$), cleaved poly (ADP-Ribose) polymerase 1 (PARP-1) (67% increase, $p<0.0001$), and tumour suppressor p53-binding protein (53BP1) ($106.5\pm66.70\%$ increase, $p<0.001$). Similarly, significant up-regulation of -H2AX was detected in neuronal cells expressing poly(GR)100 and poly(PR)100 compared to control cells (polyGR: $62.50\pm10.63\%$ increase; polyPR,$76\pm14.16\%$ increase, $p<0.0001$), confirming that DNA damage is triggered by the DRP's. Moreover, we show that NPM1 and apurinic/apyrimidinic endonuclease 1 (APE1), a core enzyme involved in base excision DNA repair (BER), co-immunoprecipitate in C9ORF72 patients, and this co-immunoprecipitation is enhanced in ALS compared to control subjects (2.3-fold change, $p<0.05$). Furthermore, we demonstrated that over-expression of NPM1 is protective in neuronal cells expressing poly(GR)100 or poly(PR)100. Significantly fewer cells were undergoing apoptosis when NPM1 was cotransfected with poly(GR)100 and poly(PR)100, as assessed using activation of caspase3 by immunocytochemistry ($p<0.0001$, $p<0.001$). We also demonstrated down-regulation of PI3K and p-eIF4G in C9ORF72 patient tissues compared to controls, implying that the AKT/PI3k pathway is inhibited in ALS motor neurons.

Conclusion: Maintaining the stability and integrity of the genome is essential for normal cellular viability, and DNA damage can arise from both endogenous and exogenous sources. In this study we demonstrate that DNA damage and activation of the DDR is triggered by the C9ORF72 repeat expansion in ALS.

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C45 THE RNA-BINDING PROTEIN, HNRNP K, FORMS A CRITICAL NEXUS BETWEEN TDP-43 PATHOLOGY AND OXIDATIVE STRESS IN ALS

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Keywords: TDP-43, hnRNP K, Nrf2

Background: Tar DNA binding protein 43 (TDP-43) belongs to the family of heterogenous nuclear ribonucleoproteins (hnRNPs) and has been identified as the major pathological protein of ALS and FTLD-U. We have previously identified heterogenous nuclear ribonucleoprotein K (hnRNP K) as a binding partner of TDP-43 (1). Analysis of spinal cord motor neurons from ALS patients revealed a substantial decrease in hnRNP K expression compared to healthy controls (1). The results suggest that hnRNP K expression is altered in ALS where TDP-43 is mutated.

Objective: The objective of the present study is to determine the role of hnRNP K in TDP-43 associated ALS and FTLD.

Methods: The cell lines used for the study were NSC-34 cells expressing wild-type (WT) or mutant (Q331K) TDP-43 and human fibroblasts derived from ALS patients harboring an M337V mutation in TDP-43 and control human fibroblasts. To investigate hnRNP K in FTLD-TDP-43 patients, we analysed the expression of hnRNP K from healthy controls and FTLD-TDP patient brain. A TDP-43 Q331K transgenic mouse model was also employed.

Results: In pre and post-symptomatic patient fibroblasts, TDP-43 M337V mutation induces attenuated protein expression of hnRNP K compared to healthy control fibroblasts. We observed a substantial decrease in the expression of hnRNP K in both detergent soluble (RIPA) and detergent insoluble (UREA) extracts in pre and post-symptomatic TDP-43 M337V patient fibroblasts compared to control fibroblasts. NSC-34 cells expressing TDP-43 Q33IK mutation also displayed attenuated expression of hnRNP K in detergent insoluble extracts. We analysed the expression of hnRNP K from healthy controls and FTLD-TDP patient brain (n=20). Using quantitative RT–PCR (qRT-PCR), we detected a significant increase in the gene expression of hnRNP K in FTLD-TDP patients relative to controls. RNA chromatin immunoprecipitation (RNA ChIP) studies revealed that hnRNP K protein binds to nrf2 transcript, with greater enrichment in patient fibroblasts expressing TDP-43 M337V mutation, compared to healthy controls (n=3). Nrf2 expression, an antioxidant protein, was attenuated in TDP-43 M337V patient fibroblasts compared to healthy controls.

Discussion and conclusion: The data demonstrates that disease-causing mutations in TDP-43 induce attenuated expression of hnRNP K gene and protein. We also identify for the first time an interaction between hnRNP K and antioxidant transcripts in ALS. The attenuated expression of Nrf2 protein in mutant TDP-43 M337V patient cells suggests an impairment in translation of antioxidant gene transcripts bound to hnRNP K, which may have deleterious consequences on antioxidant responses in TDP-43 M337V cells. The data support the idea that abnormal interactions of hnRNP K with antioxidant transcripts promotes cellular toxicity, and this may contribute to the pathogenesis in ALS where TDP-43 is mutated.

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Session 6B Nutritional Management and Metabolism

C46 DELINEATING MECHANISMS OF DYSPHAGIA IN ALS

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Keywords: bulbar, dysphagia, mechanisms

Background: Although it is well-established that swallowing impairment, or dysphagia, occurs in the majority of individuals with amyotrophic lateral sclerosis (ALS), underlying pathophysiological mechanisms leading to unsafe or inefficient swallowing have not been determined. Given the progressive nature of dysphagia in ALS, an understanding of contributing physiological mechanisms would provide a foundation for the development, evaluation and implementation of targeted management strategies to improve, maintain or prolong swallowing function and maximize oral intake and quality of life. We therefore aimed to identify mechanisms of swallowing impairment in individuals with ALS.

Methods: A sample of 26 individuals with definite ALS (El-Escorial Criterion) completed a standardized videofluoroscopic swallowing protocol. Frame-by-frame objective analyses were completed in a blinded fashion and included: Penetration-Aspiration scale (PAS), normalized residue ratio scale (NRRS), pharyngeal constriction ratio (PCR), timing and kinematic measures, swallow frequency counts and laryngeal vestibule closure status (LVC) (complete/incomplete). Swallowing safety and efficiency profiles were determined and ANOVAs were used to compare ratings across airway safety groups (safe = PAS <2, unsafe = PAS >3) and efficiency groups (inefficient = NRRS >0.07).

Results: 88% of this cohort demonstrated impairments in swallowing efficiency and 56% in airway safety. Overall profiles indicated that no patient (0%) demonstrated unsafe and efficient swallowing, however 42% demonstrated inefficient and safe swallowing. Aspiration during the swallow represented the primary time point of unsafe swallowing and identified mechanisms of aspiration included: (i) increased time to LVC (186ms vs. 335ms, $p<0.02$), (ii) incomplete LVC (67% on thin-liquid trials, 100% on paste trials), and (iii) a higher swallowing frequency (1.38 vs. 3.71, $p<0.001$). Motor responses to aspiration included 65% effective cough, 1% ineffective cough and 33% no cough response. Identified mechanisms of residue during swallowing included: longer duration to maximal hyoid elevation, and decreased pharyngeal constriction. Interestingly, we also noted a correlation between degree of vallecular reside and maximal isometric lingual pressure ($p<0.05$).

Discussion: Identified pathophysiological mechanism of aspiration pointed to the ability of the laryngeal vestibule to completely occlude and the duration of approximation or occlusion. Decreased linudal strength and pharyngeal constriction represented primary mechanisms for valleculae and pyriform sinus residue respectively. These data are highly suggestive of a temporal progression of swallowing impairment in ALS, with impairments in efficiency appearing first at the valleculae (and likely related to the early emergence of tongue weakness), then in the pyriform sinuses, and subsequently followed by the emergence of unsafe swallowing. Longitudinal studies of swallowing decline need to confirm these interesting findings.

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C47 EATING AND COGNITION ACROSS THE AMYOTROPHIC LATERAL SCLEROSIS—FRONTOTEMPORAL DEMENTIA SPECTRUM: EFFECT ON SURVIVAL

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Keywords: eating behaviour, survival, metabolism

Background: Increasingly changes in metabolism and eating behavior are recognized as affecting the process of neurodegeneration in both frontotemporal dementia and Amyotrophic lateral sclerosis, with potential effects on disease progression and survival.

Objective: To examine body mass index (BMI), caloric intake, eating behavioral changes and survival across the spectrum of ALS with and without cognitive impairment and FTD in comparison to pure behavioral variant FTD (bvFTD).

Methods: Validated questionnaires on appetite, hunger and satiety, caloric and macronutrient consumption, and eating behaviors were completed by 143 participants: 62 ALS patients (29 pure, 12 plus—cognitive impairment insufficient to meet criteria for FTD: ALS-P and 21 ALS-FTD) were compared with 56 bvFTD and 25 control subjects. Body mass index measurements were collected prospectively. Survival analyses were conducted using Cox regression analyses for both body mass index and eating behavioural changes.

Results: ALS-FTD (mean = 29.9) and bvFTD (29.2) patients exhibited increased BMI compared to controls (25.3), with ALS (26.4), with ALS-P (27.1) subjects in between. ALS-FTD (mean = 10,584.6 kJ) and bvFTD (mean = 9530.9 kJ), but not ALS and ALS-P patients, had increased food intake compared to controls. ALS-FTD and bvFTD patients exhibited increased

carbohydrate and sugar intake, whilst ALS and ALS-P, ALS-FTD and bvFTD patients demonstrated increased saturated fat intake. There is a spectrum of eating behavior changes with the greatest abnormalities in bvFTD and ALS-FTD patients, through to ALS-P, with more subtle changes in pure ALS patients. ALS patients with cognitive impairment developed changes in food preference, whilst bvFTD patients developed changes in all aspects of eating behavior. These changes in eating behavior affected survival, with the development of eating behavioral abnormalities reducing the risk of dying by three fold ($p<0.001$), after adjusting for age, cognition and diagnosis.

Conclusions: ALS is not simply a disease of decreased oral intake. Changes in eating behavior and metabolism (BMI) occur in ALS in association with increasing cognitive impairment, creating an additional component to the ALS-FTD spectrum. Eating behavioural changes suggest involvement of neuroendocrine–hypothalamic pathways in both ALS and FTD and have a protective influence against more aggressive disease states, potentially by affecting underlying pathophysiology.

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C48 DOES PERCUTANEOUS ENDOSCOPIC GASTROSTOMY LENGTHEN SURVIVAL IN PATIENTS WITH WEIGHT LOSS WHEN BULBAR FUNCTION IS PRESERVED?

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Keywords: weight loss, percutaneous endoscopic gastrostomy, survival

Objective: To examine the benefit of percutaneous endoscopic gastrostomy (PEG) in patients with weight loss despite adequate bulbar function.

Background: Weight loss in ALS correlates with reduced survival. Current practice supports PEG feeding when weight is not sustained. However, no study to date addresses whether weight loss in patients with good bulbar function confers a similarly poor prognosis and whether PEG effectively reduces weight loss and/or results in a survival benefit in these patients.

Method: We conducted a retrospective analysis of data collected prospectively in two phase 3 trials. The first, of minocycline, enrolled 412 patients with ALS, between 2003 and 2005. We included the 221 patients who had normal or near normal bulbar function, as defined by a maximum total loss of three points on the speech, saliva and swallowing questions on the ALSFRS-R (FRS b > 9). The second trial, of dexpramipexole, enrolled 943 ALS patients across 11 countries in 2011. For each cohort, we analyzed the correlation between survival and percentage weight loss; percentage weight loss as a function of the rate of decline in forced vital capacity (FVC); and the effect of PEG on weight loss and mortality.

Results: In patients with FRS b >9, weight loss of >5% over 6 months was associated with reduced survival. In the minocycline trial, median survival was 33 months when there was minimal weight loss (0–5%) and 14 months when there was ≥5% weight loss ($p<0.001$ for survival difference). The mean FVC decline (over the first 6 months of follow-up) was 9% in the minimal weight loss group, 13% in the 5–10% weight loss group and 25% in the >10% weight loss group. In patients with near normal bulbar function, PEG insertion had a non-significant effect on reducing the rate of weight loss but did not result in a survival benefit. Follow up in the dexpramipexole trial was shorter, but included more patients with PEG and confirmed the minocycline trial.

Conclusion: In patients with preserved bulbar function, weight loss ≥5% is associated with more rapid decline in FVC and increased mortality. PEG in these patients may slow weight loss but did not result in a survival benefit in either of these cohorts. These data justify caution in offering PEG to patients with preserved bulbar function on the basis of weight loss alone. The proportion of patients receiving PEG in 2011 was significantly greater than in 2005, emphasizing the importance of further study in this area.

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C49 A DECREASE IN BLOOD CHOLESTEROL AFTER GASTROSTOMY COULD IMPACT SURVIVAL IN ALS

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Keywords: gastrostomy, denutrition, cholesterol

Background: Disturbances of nutritional status represent a major prognostic factor in Amyotrophic Lateral Sclerosis. An alteration of metabolism, including lipids has been described in ALS and its relationship with survival is still controversial. The recourse of gastrostomy showed global benefit for ALS patients. However, the impact of gastrostomy on biological parameters has never been explored. The aim of this preliminary work was to evaluate the modification of biological parameters in ALS patients undergoing gastrostomy and their effects on survival.

Methods: We retrospectively collected clinical and biological data (lipid, iron metabolism, hepatic examinations etc) from ALS patients having gastrostomy at 3 times points (T0: about 9 months before gastrostomy, T1: at the time of gastrostomy, and T2: about 6 months after gastrostomy) to assess the evolution of these parameters after gastrostomy placement. We compared the values of these parameters between these 3 time points, and the

percentage of evolution before and after gastrostomy (T1–T0; T2–T1) using paired Wilcoxon test. The parameters that evolve differently before and after gastrostomy were compared to those of ALS patients without gastrostomy (controls). We assessed the relationship between biological parameters and disease progression.

Results: We collected data from 44 ALS patients and 225 controls that were similar except for site at onset and nutrition status at diagnosis. Variations of the serum concentrations of total cholesterol (p=0.0044), LDL-cholesterol (p=0.004) significantly differed before (T1–T0) versus after gastrostomy (T2–T1). We also observed a trend to a significance for LDL/HDL ratio (p=0.017). The variation of total cholesterol serum concentration after gastrostomy (T2-T1) was negatively associated with survival (p=0.0002) as well as for LDL-cholesterol (p=0.0002).

Discussion: This study showed for the first time that ALS patients fed by nearly exclusive gastrostomy had significant modifications of lipidic profiles marked by a decrease in blood cholesterol after gastrostomy conversely to what observed during the period preceding the intervention. We suggest that the type of nutrition, especially a supplementation in cholesterol in addition to gastrostomy has to be controlled by an interventional study in a large cohort of ALS patients.

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C50 GUT APPETITE REGULATORY AND METABOLIC HORMONES IN ALS: RELATIONSHIP TO BODY COMPOSITION, ENERGY EXPENDITURE AND SURVIVAL

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Keywords: appetite, metabolism, nutrition

Introduction: Some ALS patients experience anorexia in the absence of depression. The reason for the appetite loss is unknown. To date, over 20 gut- and fat-derived hormones have been identified that regulate appetite and satiety, signal fat stores, and influence metabolism via signaling to the hypothalamus. The integrity of these regulatory systems in ALS has not been investigated to date.

Methods: This was a single site pilot study (University of Kentucky) that was a component of our larger Nutrition/NIPPV study. Twenty ALS participants with clinically definite or probable ALS were enrolled. As described previously (1), Total Energy Expenditure (TEE) was measured over a 10 day period using the doubly labeled water method (DLW, University of Vermont). After an overnight fast, we measured: clinical laboratory serum analytes, BMI, ALS clinimetrics, and body composition with BIS and DXA scanning. We measured the following regulatory hormones in the fasted state using commercial kits: ghrelin, oxyntomodulin, glucagon-like peptide 1 (GLP-1), peptide YY (PYY), leptin, adiponectin, plasminogen activator inhibitor 1 (PAI-1), and resistin. We also had complete survival data for the cohort. Pearson correlation coefficients were calculated. The correlation of the fasting hormone levels with survival was examined with the univariate log-rank test and Cox models. Significance was set at $p<0.05$.

Results: The orexigenic hormone, ghrelin, correlated positively with meal times and inversely with TEE. The fat-derived hormone, leptin correlated with body fat but adiponectin, also considered to be synthesized by fat cells, correlated negatively with body fat. PAI-1 exhibited similar positive correlations with body fat. The satiety signaling hormones, GLP-1 and PYY, correlated positively with BMI and body fat whereas oxyntomodulin did not correlate with any measured parameter. Routine clinical laboratory measures and ALS clinimetrics did not correlate with any of the regulatory hormones. Although oxyntomodulin did not correlate with body composition or TEE, oxyntomodulin correlated with survival with a hazard ratio of 0.457 (p=0.025).

Conclusion: Afferent signaling of stored energy from long-term fat stores via leptin and PAI-1 appears to be intact. Short term satiety signals from the intestine, GLP-1 and PYY, correlated positively with energy stores (fat). As reported previously in non-ALS subjects, ghrelin likely stimulated appetite in ALS as indicated by correlation with self-reported meal time. Although the regulation of appetite and metabolism is exceedingly complex, the finding of the relationship of oxyntomodulin and survival requires replication and further research as a potential modulatory factor for survival in ALS.

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C51 CHANGES IN ENERGY METABOLISM IN ALS ARE ASSOCIATED WITH ALTERATIONS IN GLUCOSE AND FATTY ACID FLUX

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Keywords: energy metabolism, glucose homeostasis, adipose

Background: Studies indicate that patients with ALS have impairments in whole body physiology and energy homeostasis, with evidence that an imbalance in energy metabolism negatively influences the rate of progression of disease. By investigating energy needs in parallel with glucose and fatty acid flux, we may identify mechanisms

that underpin the association between metabolic dysfunction and the aetiology of neurodegeneration in ALS.

Objective: To determine whether changes in whole body energy needs are associated with altered capacity to utilize glucose and lipids as energy substrates in ALS.

Methods: We assessed the metabolic needs of ALS and age- and gender-matched control participants relative to their meal-associated glucose clearance (n=30 ALS, n=20 control). Separate to this, we assessed primary components of energy homeostasis (daily energy use, activity and food intake) in a preclinical mouse model of ALS ($n \geq 8$ per group). By developing a novel method to investigate cellular metabolism in intact skeletal muscle fibres, we extended our studies in ALS mice to include *in situ* analysis of glucose and lipid use in skeletal muscle in real-time ($n \geq 3$ per group).

Results: We demonstrate that the progressive increase in energy needs in ALS patients is associated with impairments in the early post-prandial clearance of blood glucose. In ALS mice, an increase in energy needs developed throughout the course of disease. Despite increasing food intake and reducing physical activity, ALS mice were not able to offset increasing energy needs, and this resulted in the rapid depletion of endogenous adipose stores. The loss of adipose coincided with increased lipid mobilization, and increased dependency and capacity for skeletal muscle to use lipid as an energy source.

Discussion and conclusion: A rise in energy needs occur alongside worsening glucose clearance in ALS patients. Higher metabolic needs and the depletion of adipose stores in ALS mice occurs in response to a failure to offset changing energy needs, and a concomitant rise in dependence on lipid as an energy source in skeletal muscle. Thus, impairments in muscle metabolic physiology likely contribute to the changing metabolic needs in ALS.

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Session 6C Neuroimaging

C52 GREY MATTER CORRELATES OF COGNITIVE DECLINE IN ALS: A MULTI-ATLAS BASED MRI STUDY

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Keywords: cognition, MRI, basal ganglia

Background: Cognitive decline (CD) and behavioral changes (BC) affect up to 50% of ALS patients. The most frequent alterations are executive, verbal memory and language dysfunction, along with apathy, disinhibition and inappropriate behavior. This supports the continuum between ALS and FTD, and brings a new perspective regarding multi-domain cerebral damage in ALS. Despite that, MRI studies looking at the structural correlates of ALS-related CD and BC are scant and have conflicting results. Multi-atlas based analysis (MABA) is a technique that relies upon a robust segmentation algorithm that enables multimodal evaluation of MRI datasets. There are very few studies that employed MABA to investigate brain damage in ALS.

Objectives: To elucidate the structural brain correlates of CD and BC, through a multimodal approach using MABA.

Methods: Forty-seven non-demented C9orf72 negative Brazilian ALS patients (25 males, mean age 56 ± 9 years, mean ALSFRS-R 35 ± 7, mean disease duration 37 ± 49 months, mean education 10 ± 5 years) underwent a proper clinical evaluation and detailed neuropsychological assessment. Ten patients were diagnosed with CD (ALSci) and 13 with BC (ALSbi) according to current criteria. Two patients were diagnosed with concomitant CD and BC. All participants underwent an MRI acquisition on a 3T scanner on the same day. Thirty-eight healthy controls also underwent MRI scans. We obtained high resolution T1-weighted images in order to assess grey matter integrity. The FreeSurfer suite was employed to investigate cortical thickness alterations, and deep grey matter volume was evaluated using T1 multi-atlas approach. For statistical analysis, MRI parameters of the ALSci group were compared with two control groups: (i) the same number of age, gender, education and ALSFRS-R paired ALS patients without cognitive alterations (ALSni); and (ii) Age- and gender-matched healthy controls (n=20). We followed the same procedure for the ALSbi group (healthy controls n=23). False discovery rate (FDR) was employed for multiple comparisons, with corrected $p \leq 0.05$ considered significant.

Results: The ALSci group presented thinner cortical thickness in extramotor areas compared to healthy controls, mostly on the left hemisphere. Regarding deep grey matter, ALSci group had reduced left thalamus (p=0.005) and bilateral amygdalae (left p=0.005, right p=0.017) volumes when compared to ALSni patients and healthy controls, and bilateral volume reduction of limbic areas (left p=0.005, right p=0.017) when compared to healthy controls. Except for right limbic volume, all aforementioned deep grey matter structures volumes correlated with ALS-CBS scores in the ALS cohort, Bonferroni corrected. There were no changes of cortical thickness or deep grey matter volume in the ALSbi group.

Discussion and conclusion: Our results suggest that deep grey matter damage is specifically related to CD in ALS patients. In particular, thalami and amygdalae seem to play an important role in its occurrence.

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C53 CORTICAL PROFILE OF C9ORF72 GENE EXPRESSION ASSOCIATED WITH CORTICAL THINNING IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: C9orf72, gene expression, MRI

Background: Whole-genome sequencing has identified the hexanucleotide repeat expansion in the C9orf72 gene, as a highly penetrant mutation in 37% of familial amyotrophic lateral sclerosis (ALS) and 25% of familial frontotemporal dementia (FTD) cases (1,2). Furthermore, ALS patients carrying the C9orf72 mutation have been shown to exhibit extensive cortical and subcortical involvement (3,4). The pathogenic mechanism underlying C9orf72-associated ALS, however, remains unclear.

Objectives: Gene-associated pathogenic mechanisms are often investigated using mouse models. Here we adopt a novel approach testing normative expression levels of the C9orf72 gene across the cortex for a possible association with ALS-specific regional cortical thinning effects.

Methods: Anatomical T1-weighted MR images were acquired from 184 sporadic ALS patients and 113 healthy controls on a 3T MRI scanner. For all participants the cortex was parcellated into 57 distinct regions and a cortical profile of regional thickness values per subject was obtained. The ALS cortical thinning profile was then

defined by the average region-wise patient-control differences. An average cortical C9orf72 gene expression profile was extracted from the Allen Human Brain Atlas (5), including RNA microarray data collected from post-mortem brains of six human donors with no history of neuropsychiatric or neuropathological disorders. Gene expression levels were normalized across samples and across donors. The relationship between cortical thinning and C9orf72 expression was evaluated using Pearson's correlation analysis. Selecting 10,000 random genes from the total of 20,737 genes available in the Allen Human Brain Atlas provided the null model to test for statistical significance.

Results: We observed a significant correlation between normative levels of C9orf72 gene expression and ALS-associated effects of cortical thinning ($r = -0.41$, $p=0.02$), indicating regions with lowest C9orf72 expression to be more severely affected in ALS. Occipital and parietal regions showed highest levels of C9orf72 gene expression and no cortical thinning, while precentral and entorhinal regions exhibited the strongest cortical thinning coinciding with lowest levels of C9orf72 gene expression.

Discussion and conclusions: Our observation that C9orf72 gene expression is correlated with cortical thinning suggests C9orf72 gene expression to be involved in the pathogenic mechanism underlying ALS. Cortical regions with relatively low C9orf72 gene expression showing strong cortical thinning may indicate that lower C9orf72 gene expression levels render regions more vulnerable to the disease.

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C54 FUNCTIONAL AND STRUCTURAL CONNECTIVITY IN ALS – INSIGHTS FROM MRI CONNECTOME ANALYSES AND TMS

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Keywords: MRI, Transcranial magnetic stimulation, pathophysiology

Background: Magnetic resonance imaging (MRI) and Transcranial magnetic stimulation (TMS) are commonly utilized approaches in evaluating pathophysiological processes underlying amyotrophic lateral sclerosis (ALS). We explored a novel aspect of MR imaging, utilizing Connectomics-based analysis of resting fMRI data to explore functional pathophysiological changes in ALS. In addition to this we also analysed MRI grey matter volume changes. Finally, we correlated MRI findings with a novel threshold tracking TMS approach which has previously been shown to identify early cortical dysfunction in ALS patients.

Objectives: To explore functional MRI connectivity changes and correlate these changes with a novel threshold tracking TMS technique to better understand the pathophysiological processes in ALS.

Methods: A prospective study was undertaken on 20 ALS patients and 20 age-matched controls. Resting functional connectomes were mapped based on BOLD sequences generated from functional MRI and were analyzed using a graph theoretical approach. Global brain (path length; a measure of integration and clustering coefficient; a measure of interconnectivity of a network) and local regional measures (nodal degree, a measure of participation within the brain network) were computed. Furthermore, grey matter volume was also measured from a T1 sequence to assess structural changes in ALS. Finally, threshold tracking TMS was undertaken to derive measures of cortical excitability, including the short interval intracortical inhibition (SICI).

Results: Connectome analysis revealed an overall increased connectivity with a higher clustering coefficient in ALS patients when compared to controls ($p=0.04$). Interestingly, reduced nodal degree was seen in the frontal regions of the brain and an increased nodal degree was seen in the posterior occipital-temporal brain regions. Grey matter volume analysis revealed reduced volumes in the frontal and prefrontal regions in ALS patients when compared to controls ($p<0.05$). TMS studies suggested cortical dysfunction in ALS patients, with a reduced SICI of $1.4 \pm 2.4\%$. Multivariate analysis between MRI and TMS revealed that SICI significantly correlated with overall nodal degree in ALS patients ($F(1, 15) = 1269.6$, $p=0.02$). There was no significant correlation between SICI and overall grey matter volume.

Discussion: A novel MRI technique of functional connectivity highlights region specific abnormalities in ALS patients. Furthermore these changes correlate with a functional neurophysiological parameter of cortical hyperexcitability, which has previously been shown to be an early marker of cortical dysfunction. These techniques may aid in evaluating the complex pathophysiological processes underlying ALS.

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C55 THE PROGRESSION OF CEREBRAL PATHOLOGY IN ALS: A SIX-MONTHLY MULTI-MODAL MRI STUDY OVER TWO YEARS

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Keywords: MRI, multimodal, progression

Background: The gold standard measure of disease progression is the revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R), based on broad measures of disability remote from histopathological changes. MRI is uniquely able to assess brain structure and function, and provide a deeper understanding of *in vivo* evolution of cerebral pathology, linking cellular and system dysfunction in ALS.

Objectives: To perform multi-modal analysis of structural and functional MRI in a cohort of patients in which data were acquired at six-monthly time points for two years.

Methods: Five time point T1-weighted and resting state fMRI (rs-fMRI) data were available in 16 patients (13 ALS, 3 PLS, mean age at first scan = 60 ± 12 years, mean ALSFRS-R = 34 ± 5). Diffusion-weighted data was available for a subset of eleven patients. Average interval between time points was 6.4 ± 0.6, 6.7 ± 0.5, 6.5 ± 1.5, and 7.2 ± 1.6 months. Grey matter changes were assessed using voxel-based morphometry (FSL-VBM) and shape analysis of sub-cortical structures (FSL-FIRST). White matter changes were assessed using tract-based spatial statistics (TBSS) of diffusion tensor imaging metrics. Rs-fMRI functional connectivity (FC) was investigated via independent component and dual regression analyses, with a VBM voxelwise regressor included in the FC analysis to control for confounding effects of grey matter decline. Linear changes with time and brain changes correlated with ALSFRS-R decline were studied.

Results: VBM analysis revealed widespread grey matter decline for both analyses, with overlapping areas covering precentral gyri and posterior cingulate cortex. Shape analysis of sub-cortical structures revealed progressive local atrophy of the thalamus, caudate, and pallidum bilaterally, and for the right putamen, hippocampus and amygdala. No significant white matter changes were found over time, or in relation to ALSFRS-R decline. Rs-fMRI analysis revealed FC decreases between the sensorimotor resting state network and the frontal pole, between a network comprising both thalami, and an area in the visual cortex both over time and in relation to ALSFRS-R decline. FC increases between the left fronto-parietal network and a region in the left primary motor cortex were seen for both statistical approaches.

Discussion and conclusion: In keeping with findings from a larger cohort with only two time points (1), grey matter MRI measures are the most prominent surrogate markers of disease progression. The FC results reveal a picture of regional decreases and increases noted in other studies (2), compatible with compensatory responses to disintegration of motor and frontal projection networks.

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C56 DEVELOPMENT OF AN AUTOMATED MRI-BASED DIAGNOSTIC PROTOCOL BASED ON DISEASE-SPECIFIC PATHOGENOMIC FEATURES IN AMYOTROPHIC LATERAL SCLEROSIS: A QUANTITATIVE DISEASE-STATE CLASSIFICATION STUDY

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Keywords: biomarker, classification, binary logistic regression

Introduction: Despite advances in quantitative neuroimaging, the diagnosis of ALS remains clinical and MRI-based biomarkers are not currently used to aid the diagnosis. The objective of this study is to develop a robust, multi-modal classification protocol and assess its diagnostic accuracy in an independent, early-stage data set.

Methods: 147 participants (81 ALS patients and 66 healthy controls (HC)) were divided into a training sample (75%) and a validation sample (25%). Patients and healthy controls in the validation sample underwent longitudinally follow-up imaging. Discriminating input features were selected based on group comparisons between patients and controls in the training sample to compute a binary regression. Tract-based white matter alterations were explored based on fractional anisotropy (FA), radial (RD), mean (MD), and axial diffusivity (AD) indices. Grey matter alterations were explored using voxel-based morphometry. After removing age-related variability, indices of grey and white matter integrity were included in a cross-validated binary logistic regression model to determine the probability of individual scans indicating ALS. The resulting algorithm was then validated in an independent sample and further evaluated based on the follow-up scans of these participants. The sensitivity, specificity and accuracy of this approach were calculated for each sub-cohort separately.

Results: Based on the group comparisons, the following brain structures were selected as input features for the classification algorithm: the mean grey matter density of the precentral gyrus, the mean FA and RD of the genus, the body and the splenium of the corpus callosum, the inferior corticospinal tracts, the cerebral peduncles, the posterior and anterior limbs of the internal capsule, the inferior coronae radiata and the superior corona radiata. Using a cut-off score of 50% probability, the model was able to discriminate ALS patients and HC with good sensitivity (80.0%) and moderate accuracy (70.0%) in the training sample and good sensitivity (85.7%) and accuracy (78.4%) in the independent validation sample.

Conclusions: This study seeks to translate advances in ALS biomarker research into pragmatic clinical applications by outlining an approach for individual-data interpretation. The advantage of using a binary logistic regression approach is that it results in a probability score which can be integrated into clinical decision-making in the diagnostic phase of the disease.

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C57 DATA-DRIVEN MODELLING OF DIFFUSION MRI CHANGES IN ALS INDICATES EVOLUTION OF DISTAL PRIOR TO PROXIMAL CORTICOSPINAL TRACT PATHOLOGY

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Keywords: disease progression, MRI, event-based model

Background: A key aim of medical science is modelling patterns of disease progression; these patterns increase understanding of the disease, and help construct staging systems that assist diagnosis and treatment. Within ALS disease progression modelling, there is a need to integrate clinical observation-based staging systems such as (1), which suffer from low temporal resolution, with 'unbiased' staging of biomarkers. To this end, we have adapted and extended an event-based model (EBM) for ALS from previous work in Alzheimer's disease (2,3). Unlike traditional models of disease progression, event-based models do not rely on a priori staging of patients but extract the event ordering directly from the data, thus minimizing subjective bias. In MR imaging, fractional anisotropy (FA) derived from diffusion tensor imaging is an obvious candidate to test the hypothesis that imaging events can be staged in the EBM.

Objectives: Using modern and historical amyotrophic lateral sclerosis (ALS) datasets comprising diffusion MRI data, we used a novel event-based model to analyse the likely ordering of these biomarkers in the progression of ALS.

Methods: The historical dataset was derived from a cross-sectional sample of 28 ALS patients and 26 matched controls (4), and the modern dataset was similarly derived from 23 ALS patients and 23 matched controls (5). No ALS patients were classified as having ALS-FTD. All the diffusion-weighted images were co-registered and then normalized into MNI space using ANTs 2.1.0, and the JHU label atlas was used to derive the FA values for specific tracts.

Results: The most likely order of progression showed that FA changes in the lower aspect of the corticospinal tract (CST) occur at an early stage of disease evolution, with changes in the upper aspect occurring at a later stage. This result was found individually in both datasets, as well as when combining them.

Discussion: This proof-of-principle study shows that data-driven models of ALS progression are feasible. Our results suggest very robustly that damage to CST starts in the lower aspect. Nevertheless, some important limitations must be discussed. The small sample size may have biased our results. We have tried to address this issue by assessing how the results varied across two separate datasets, both individually and combined. While the CST results were consistent across the entire process, results for other regions such as the corpus callosum were less constant, suggesting that the biomarker ordering in the wider population may diverge from this sequence. Future studies on larger datasets are warranted.

Conclusion: These findings may provide support for the 'dying-back' hypothesis of motor neurone degeneration.

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Session 7A Epigenetics and Genomics

C58 EPIGENETIC PATHWAYS TO NEUROPSYCHIATRIC AND NEUROLOGICAL DISEASE

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Keywords: epigenetics, genetics, genomics

Contemporary research aimed at exploring the etiology of neurological and neuropsychiatric disease has focused primarily on DNA sequence variation, with considerable success. Increasing knowledge about the biology of the genome also highlights an important role for epigenetic variation in human health and disease; recent methodological advances mean that epigenome-wide association studies (EWAS) are now feasible for complex disease phenotypes. In this talk I will present on-going work from our group aimed at identifying epigenetic variation associated with a diverse range of brain disorders including: schizophrenia, autism and Alzheimer's disease. I will describe an analysis of dynamic DNA modifications (5 methylcytosine and 5 hydroxymethylcytosine) across human brain development, highlighting how the prenatal period is a time of considerable epigenomic plasticity in the brain, and the importance of neurodevelopmentally-dynamic loci in disorders affecting the mature brain. I will also describe the impact of genetic variation on the epigenome, presenting our recent analysis of DNA methylation quantitative trait loci (mQTLs) in a large collection of fetal and adult brain samples. In particular, I will highlight how epigenetic variation can be used to refine signals from genetic studies of complex disease. Finally, I will outline some of the issues related to epigenetic epidemiological studies of brain diseases and explore the feasibility of identifying peripheral biomarkers of disease phenotypes manifest in inaccessible tissues such as the brain.

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C59 EPIGENETIC MODELING AND THERAPEUTIC TARGETING OF THE EXPANDED C9ORF72 LOCUS

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Keywords: C9ORF72, iPSC, Epigenetics

Background: The most common known genetic cause of both ALS and FTD is a repeat expansion mutation within the C9ORF72 gene. The mutation leads to partial epigenetic repression of the locus, yet mutant RNAs and dipeptide repeat proteins (DPRs) are still produced in sufficient quantities to confer neurotoxicity. Expanded C9ORF72 alleles are enriched with repressive histone tail modifications, DNA hypermethylation of the promoter, and reduced transcription rates. Notably, a higher degree of epigenetic repression results in a modestly attenuated phenotype. The degree to which toxic C9ORF72 RNAs, DPRs, and loss of gene function confer pathology remains unresolved. Furthermore, while nuclear stress and disruption of nucleocytoplasmic trafficking (NCT) are key features of C9ALS/FTD, it remains to be determined whether nuclear import, nuclear export, or both are primarily affected.

Objectives: The primary goals of our ongoing studies are to develop and characterize appropriate motor neuron model systems in order to (i) investigate epigenetic features of the C9ORF72 repeat expansion mutation, (ii) conduct therapeutic screening campaigns and (iii) identify relevant cellular phenotypes to advance therapeutic development.

Methods: From accessible patient tissues, we used cellular reprogramming to generate motor neurons that harbor the C9ORF72 expansion mutation. We then used bisulfite pyrosequencing and hydroxymethylation sensitive restriction digest and PCR to assess 5 methylcytosine (5mC) and 5 hydroxymethylcytosine (5hmC) within the C9ORF72 gene promoter. We carried out a C9ORF72 gene expression-based screen using reprogrammed motor neurons to interrogate an epigenetic small molecule library and developed a fluorescent reporter protein to quantify nucleocytoplasmic trafficking kinetics.

Results: Our data show that DNA hypermethylation of the C9ORF72 promoter is recapitulated in reprogrammed motor neurons, indicating they are a relevant epigenetic model system. Furthermore, we identified a novel epigenetic feature of the C9ORF72 expansion, 5hmC, which is present both in reprogrammed motor neurons and clinical samples. We found inhibitors of jumonji domain histone lysine demethylases (JMJ-KDMs), which carry out the catalytic activity of most histone demethylases, reduce C9orf72 gene expression levels.

Discussion and conclusions: Our finding that 5hmC is enriched within the expanded C9ORF72 promoter indicates that active DNA demethylation may prevent more complete epigenetic silencing of the locus. While seemingly unrelated to this mechanism, hits from our epigenetic small molecule screen identified jumonji domain inhibitors that reduce C9ORF72 expression, and may therefore hold therapeutic value. In light of the emerging consensus that dysregulation of NCT is the cellular phenotype at the heart of C9ALS/FTD pathophysiology, therapeutic agents capable of restoring normal transport kinetics are likely to have the shortest path towards clinical translation. We hypothesize that epigenetic regulation of the expanded locus represents a

promising therapeutic strategy to reduce the load of toxic RNA and DPR species and restore cellular phenotypes, including NCT.

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C60 CHANGES IN EXPRESSION LEVELS OF HOMEOBOX GENES AND TRANSTHYRETIN IN PATIENTS WITH C9ORF72 REPEAT EXPANSIONS

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Keywords: C9ORF72, transthyretin, homeobox genes

Background: A hexanucleotide repeat expansion in chromosome 9 open reading frame 72 (C9ORF72) represents the most common genetic cause of motor neuron disease (MND) and frontotemporal lobar degeneration (FTLD). Much remains unknown about the mechanism underlying C9ORF72-related diseases, and importantly, no validated biomarkers exist for diagnostic purposes, as prognostic indicators, or to monitor drug effects.

Objective: We performed a genome-wide brain expression study to reveal the underpinnings of diseases linked to a repeat expansion in C9ORF72 and to identify potential biomarkers.

Methods: From the Mayo Clinic Florida Brain Bank, we selected 32 patients with a pathological diagnosis of FTLD and/or MND who harbored C9ORF72 repeat expansions, 30 patients with FTLD and/or MND without repeat expansions, and 20 control subjects without neurological diseases, for whom frozen cerebellum and/or frontal cortex was available. In addition to genome-wide expression arrays, we used genome-wide methylation arrays, methylation-sensitive restriction enzyme digests, quantitative real-time PCR, cell culture experiments, Western blotting, and enzyme-linked immunosorbent assays.

Results: Excitingly, our analysis revealed a significant up-regulation of homeobox (HOX) genes in C9ORF72 expansion carriers. This up-regulation of genes that play a vital role in neuronal development was most profound in the cerebellum, when comparing expansion carriers to patients without an expansion (top hit: homeobox A5 (HOXA5), p=4.09e – 14). Additionally, we observed an up-regulation of transthyretin (TTR, p=4.98e – 04), an extracellular protein that is involved in neuroprotection. Previously, we investigated the levels of known C9ORF72 transcript variants and intron containing transcripts in brain tissue specimens of 56 C9ORF72 expansion carriers, 31 FTLD and/or MND patients without expansions, and 20 control subjects. In this cohort, we next confirmed that HOXA5 and TTR transcript levels were higher in our C9ORF72 expansion carriers than in patients without expansions or in control subjects ($p \leq$7.69e – 05). Interestingly, we also noticed that HOXA5 and TTR transcript levels were associated with C9ORF72 variant 2 transcript levels (overall: $p \leq$2.21e – 06) as well as with intron containing transcript levels (expansion carriers: $p \leq$0.003), suggesting that disease-related changes in those transcripts may have triggered the up-regulation of HOXA5 and TTR. Furthermore, for HOXA5, we uncovered associations with dipeptide-repeat proteins aberrantly translated from the repeat expansion ($p \leq$0.0002) and with methylation levels of the C9ORF72 promoter ($p \leq$0.001). For TTR, on the other hand, we showed that changes in transcript levels were reflected by changes in protein levels, both in the cerebellum (p<0.05) and cerebrospinal fluid (p=0.0001).

Discussion and conclusions: Our identification of genes involved in developmental processes and neuroprotection sheds light on potential compensatory mechanisms influencing the occurrence, presentation, and/or progression of C9ORF72-related diseases. Moreover, given that TTR contributes to neurodegenerative diseases and its protein levels may be changed in cerebrospinal fluid, we suggest that TTR requires further study as a potential biomarker for C9ORF72-related diseases.

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C61 A GENE SIGNATURE FOR AMYOTROPHIC LATERAL SCLEROSIS ASSOCIATED WITH TDP-43 PATHOLOGY

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Keywords: biomarker, TDP-43, gene expression profiling

Background: A prognostic biomarker for amyotrophic lateral sclerosis (ALS) does not exist. Current measures are observational but not predictive. The majority of ALS is characterised by cytoplasmic TDP-43-positive proteinaceous inclusions within motor neurons. Mutations in TARDBP, the gene encoding TDP-43, cause ALS, suggesting a key role for this protein.

Objectives: To establish a molecular signature of TDP-43 pathology which informs ALS biology and provides a prognostic biomarker.

Methods: We conducted immunohistochemistry and profiled total RNA from ALS-motor neurons. Chosen patients had TDP-43-positive disease, but were from disparate mutational backgrounds. Expressed transcripts which significantly tracked counts of proteinaceous inclusions within motor neurons (Spearman rank correlation, $p<0.01$) were selected. To better characterise upstream molecular pathogenesis, these tracking transcripts were used as seeds in a larger sample set ($n=17$) to identify co-expressed transcripts and so to generate networks. Networks help to define sets of genes with a common function. Networks were evaluated for enrichment (Fisher's exact test, $p<0.05$) with prognostic genes in motor neurons ($n=17$) and lymphoblastoid cells, and for genes carrying ALS-susceptibility variants with genome-wide association. Prognostic genes were identified by Spearman rank correlation with disease duration ($p<0.05$) in motor neurons; and by differential expression (Wilcox rank-sum test, $p<0.05$) between cells from patients with short (<2 years, $n=18$) and long (>4 years, $n=8$) disease duration in lymphoblastoid lines. As a control, enrichment of prognostic and susceptibility genes was assessed in genes expressed specifically in non-diseased motor neurons.

Results: We identified 83 transcripts that closely tracked pathology-load in ALS-motor neurons; co-expression analysis developed these into 82 network modules. Sixteen modules are enriched with prognostic genes in motor neurons and lymphoblastoid cells. One module of 65 genes is disproportionately enriched with prognostic genes in lymphoblastoid cells ($p=5.47E-22$). Based on the detection of prognostic expression differences in a peripheral tissue, we chose this module for assessment as a potential biomarker. Significant enrichment with ALS-susceptibility genes ($p=0.01$) within our candidate biomarker module suggests an upstream role in neurodegeneration. Remarkably, addition of genes with similar function to the biomarker module improves enrichment with prognostic and susceptibility genes, suggesting the function of this module is important to ALS-pathogenesis. The biomarker module is enriched for neuroinflammatory genes ($p<0.05$). Genes expressed specifically in non-diseased motor neurons do not show similar enrichment at any stage, suggesting our biomarker module carries an ALS-specific signal.

Conclusion: Our biomarker module provides insight into ALS and bridges the gap from neuropathology to clinical severity. For the first time, we identify a potential link between neuroinflammation and TDP-43 pathology.

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Session 7B Symptomatic Treatments

C62 THE CANALS STUDY: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, MULTICENTRE STUDY TO ASSESS THE SAFETY AND EFFICACY ON SPASTICITY SYMPTOMS OF A CANNABIS SATIVA EXTRACT IN MOTOR NEURON DISEASE PATIENTS

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Keywords: therapy; spasticity, cannabinoids

Background: Spasticity is one of the major determinants of functional loss and decline in quality of life in ALS and other motor neuron disease (MND) patients. In recent years, several clinical trials have tested the efficacy of cannabis on spasticity in multiple sclerosis.

Objectives: The study's primary aim was to evaluate the safety, tolerability and efficacy of a *Cannabis sativa* extract medium-term treatment (6 weeks) to improve spasticity in MND patients.

Methods: 60 consecutive patients fulfilling specific inclusion criteria were randomized, double blinded and allocated to receive a cannabis extract oral spray or placebo. The primary end-point was improvement in the 5-points modified Ashworth Scale (MAS). Secondary End-points: spasticity, spasm frequency and sleep disruption (0–10 NRS score); Function: walking ability, functional scores (ALSFRS-R); pain (0–10 NRS score). The Global Impression of Change (GIC) for the patient, carer and clinician.

Results: The study drug was well-tolerated and none of the patients included withdrew from the study. We observed a positive trend for improvement of all outcome measures in the active drug arm compared to the placebo group, which reached statistical significance for the MAS mean score (p=0.013) and pain NRS (p=0.013). Patients' GIC demonstrated a significant subjective improvement in 55% of subjects (p=0.001).

Conclusions: Our pilot study suggests that cannabinoids may represent a valuable option for spasticity treatment in MND patients. Moreover, it may have additional beneficial effects such as pain relief. Further studies are needed in order to confirm our results and to explore the potential neuroprotective effects of cannabinoids.

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C63 AEROBIC EXERCISE THERAPY IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS (FACTS-2-ALS): A RANDOMIZED CLINICAL TRIAL

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Keywords: exercise, quality of life, multidisciplinary care

Background: Weakness caused by motor neuron degeneration in ALS may result in avoidance of physical activity resulting in cardiovascular deconditioning and disuse weakness. This may be reversed by including aerobic exercise therapy (AET) in a multidisciplinary care program.

Objectives: To examine the effects of AET versus usual care (UC) on QoL, global function, social participation, strength of leg muscles and lung function in ALS.

Methods: In a multicenter, assessor-blinded, randomized clinical trial, patients with ALS were randomly assigned to AET or UC. AET consisted of a 16-week individually tailored aerobic exercise programme on a cycle ergometer and a stepboard, three times a week: twice at home and once in clinic. A biphasic randomization model with postponed information was used. First, patients were asked to participate in a longitudinal study including four measurements (first permission): at baseline (t=0), 4 months (t=1), 7 months (t=2), and 10 months (t=3) respectively. Second, patients were randomized: patients randomized to AET were asked to sign the informed

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consent (second permission). Patients randomized to UC did not receive additional information to prevent disappointment of withholding treatment. The primary endpoint was change in QoL (ALSAQ-40). Secondary endpoints were change in global function (ALSFRS-R), social participation (Sickness Impact Profile, social dimension), muscle strength of legs (hand-held dynamometry) and lung function (FVC %) assessed by two linear mixed models adjusted for baseline inequalities. Per-protocol (PP) analysis was performed including patients who attended >75% of the training sessions and were not lost to follow-up at 10 months.

Results: 149 patients met the eligibility criteria, 15 were enrolled in another intervention, 77 declined to participate (first permission). Fifty-seven participants were randomized; 27 to AET and 30 to UC. Nine patients declined to start AET (second permission) due to alternative activities or fast disease progression. Intention-to-treat analyses showed that AET did not have a significant effect on any of the outcome measures compared to UC. Ten of 18 patients who received AET were able to attend 75% of the sessions with sufficient follow-up. PP-analyses showed significantly less deterioration in ALSFRS-R score (slope −0.45, vs −1.09; p=0.015) and FVC % (slope −0.76 vs −2.29; p=0.016) compared to UC. Patients who completed ≥75% of AET sessions had a less steep ALSFRS-R slope at inclusion (0.24 vs 0.51), less bulbar symptoms (10% vs 35%), and higher baseline physical activity/day (4.2 vs 2.4 METs/day).

Conclusions and recommendations: This RCT shows that AET has a beneficial effect only in a selected group of ALS patients with a relatively slow disease progression, a spinal onset and a higher physical activity per day at baseline. In this group, AET can preserve global function and lung function. Further research should focus on the specific factors that contribute to this beneficial effect to optimize patient selection.

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C64 MEDITATION TRAINING FOR PEOPLE WITH AMYOTROPHIC LATERAL SCLEROSIS: A RANDOMIZED CLINICAL TRIAL

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Keywords: quality of life, mindfulness, meditation

Background: There is a lack of studies about psychological interventions for the promotion of well-being in people with Amyotrophic Lateral Sclerosis. We aimed to test the efficacy of an ALS-specific mindfulness-based intervention on the improvement of quality of life.

Methods: We conducted a randomized, open-label and controlled clinical trial of the efficacy of an ALS-specific meditation program in promoting quality of life. Adults who received a diagnosis of ALS within 18 months were randomly assigned to an 8-week meditation training (based on the original mindfulness-based stress reduction program and tailored for people with ALS) or to the usual care. Quality of life, assessed with the ALS-Specific Quality of Life Revised (ALSSQOL-R), represented the primary outcome, while secondary outcomes included anxiety and depression, assessed with the Hospital Anxiety and Depression Scale (HADS), and specific quality of life domains (Negative Emotion, Interaction with People and the Environment, Intimacy, Religiosity, Physical Symptoms and Bulbar Symptoms, all factors of the ALSSQOL-R). Participants were assessed at recruitment and after 2, 6 and 12 months. The efficacy of the treatment was assessed on an intention-to-treat basis in a linear mixed model. Study protocol was registered in ISRCTN registry, with the ID no. 88066803.

Results: One hundred participants were recruited between November 2012 and December 2014. Over time, there was a significant difference between the two groups in term of quality of life (β=0.24, p=0.015, d=0.89). Significant differences between groups over time were also found for anxiety, depression, negative emotions, and interaction with people and environment.

Conclusion: An ALS-specific meditation program is beneficial to quality of life and psychological well-being of people with ALS.

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C65 EARLY TREATMENT WITH NIPPV: FACTORS AFFECTING COMPLIANCE OVER TIME

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Keywords: NIPPV, compliance, respiratory

Background: There is evidence that non-invasive positive pressure ventilation (NIPPV) improves both survival and quality of life in ALS. There has been little work, however, on optimizing the timing of this intervention.

Objectives: To prospectively examine NIPPV compliance when started early in the course of the disease.

Methods: Seventy-three ALS patients with a forced vital capacity (FVC) > 50% were enrolled in the multi-center pilot study of Early Nutrition and NIPPV. Patients with a FVC over 80% at baseline were initiated on NIPPV when their FVC was 75–85% (Group 1 – Early intervention). Patients with a FVC between 50% and 80% were started when their FVC was 45–55% (Group 2 – Standard of Care). NIPPV compliance was defined as > 4 hours of use on 60% of days based on computer downloads. Patients were followed over 12 months.

Results: Of the 73 participants in the NIPPV arm of the study, 57 subjects were offered NIPPV (36 in Group 1 and 21 in Group 2). Data from downloads were available from 47 of the participants. By week 4 after initiation of NIPPV, the compliance rate was 53.3% for Group 1 and 70.6% for Group 2. In Group 1, compliance steadily increased after 84 days on NIPPV so that after 364 days, there was 80% compliance. In Group 2, compliance was higher at all times and remained greater than 70% after 140 days. In those subjects who were noncompliant at 28 days, 69.2% (9/13) remained noncompliant until death while 15.4% eventually became compliant; 15.4% became compliant only at terminal stages of disease. In those subjects in Group 1 who were compliant at 28 days, 81.3% remained compliant until death while 12.5% were compliant until the last time point prior to their death and only 1 subject became noncompliant over the course of follow-up. In Group 2, 91.7% (11/12) of subjects compliant at 28 days remained compliant over the course of follow-up while only one subject became noncompliant at the final visit. In Group 2, subjects who were noncompliant at 28 days, 60% remained noncompliant while 40% became compliant over the course of the study. The factors that correlated with compliance over time are being analyzed.

Discussion and conclusion: For both groups, initial compliance was maintained over the course of the study while those subjects who were non-complaint tended to remain so over the course of follow-up. There was an overall increase in compliance over time in both groups. This data supports the ability of asymptomatic patients to comply with NIPPV earlier in the course of the disease in order to potentially slow the rate of respiratory decline and to retain lung volumes.

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Session 7C Mouse Models

C66 PHENOTYPIC CHARACTERIZATION OF A NEW CHMP2BINTRON5-BASED TRANSGENIC MOUSE THAT DEVELOPS HISTOLOGICAL AND BEHAVIOURAL FEATURES OF AMYOTROPHIC LATERAL SCLEROSIS AND FRONTOTEMPORAL DEMENTIA

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Keywords: CHMP2Bintron5 mouse model, FTD/ALS, phenotyping

Background: Since the discovery of a gene involved in both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), many studies focus on the idea of genetic and pathophysiological continuum between these two neurodegenerative diseases. Indeed, 50% of FTD patients also develop motor symptoms (1). Moreover, about 15% of ALS patients concomitantly develop FTD. Chmp2B was the first mutated gene identified in both FTD and ALS patients. Chmp2b encodes a protein involved in endocytic pathways, intraluminal vesicles formation, virus budding and cytokinesis. The main form of Chmp2B mutant, found in FTD patients, is called Chmp2Bintron5. This mutation leads to the c-terminal truncation of 36 last amino acids (2). To date, the mechanisms leading to neuronal dysfunctions linked to Chmp2B mutant are poorly understood.

Objective: Study the clinical and the physiopathological continuum between ALS and FTD using a novel mouse model, which expresses CHMP2Bintron5 mutant in neurons.

Methods: Using complementary biochemical, histological and behavioural techniques we have characterized a newly generated transgenic mouse line expressing CHMP2Bintron5 mutant under control of the Thy1.2 promoter (3).

Results: The CHMP2Bintron5 mice have decreased survival and show progressive neurodegenerative changes leading to motor and behavioural alterations. They show a strong expression of the mutant protein in neurons of the brain and the spinal cord, especially in the anterior cortex and in motor neurons. This expression is associated with a gliosis and the presence of P62, ubiquitin and Chmp2Bintron5-positive inclusions. The motor phenotype recapitulates several aspects of human ALS. Homozygous mutants exhibit severe and early locomotor impairments as attested by a decrease of rotarod performance and grip strength, and gait abnormalities at 2 months of age. These impairments appear from 15 months of age in hemizygous mice and develop toward a final paralysis associated with muscle denervation, as assessed by electromyography. We further show behavioral defects relevant for FTD such as stereotypies (repetitive rearing, excessive grooming), food intake abnormalities and anxiety decrease.

Discussion: Here, we report the generation of a Transgenic line expressing a human ALS/FTD-causing mutation that reproduces part of the ALS-FTD symptoms. Our data provide robust *in vivo* evidence of neurodegenerative mechanisms driven by the expression of the CHMP2Bintron5 mutant. Next, compared with data obtain in ALS or FTD mice models, our data allowed us to identify common or specific mechanisms in the two diseases.

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C67 AAV9-MEDIATED C9ORF72 EXPERIMENTAL MODELLING OF ALS/FTD IN MICE

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Keywords: C9orf72, mouse model, AAV

Background: Expansion of a hexanucleotide repeat G4C2 in the non-coding region of chromosome 9 open reading frame 72 (C9orf72) is the most common genetic cause of ALS associated, in particular, with frontotemporal dementia (FTD). Growing evidence suggests that C9orf72 repeat expansions also contribute to a wide spectrum of neurodegenerative diseases such as

Alzheimer's, Huntington's, multiple sclerosis and Parkinson's disease. Approximately half of the non-pathogenic C9orf72 alleles possess two G4C2 repeats and the remaining half ranges from 2 to 25 repeats. The pathogenic expanded repeat length, on the other hand, varies from tens to thousands. The prevalence of C9orf72 induced ALS is quite high, and it appears to be involved in up to 40% of familial cases. This makes it the most common form of motor neuron disease discovered to date. Despite its prevalence, the mechanism(s) by which C9orf72 repeat expansion cause pathology are poorly understood.

Objectives: Assessment of the effects of hexanucleotide repeat expansions *in vitro* and *in vivo* via AAV9 mediated expression of increasing numbers of repeat expansions. Here, we assess the use of AAV9 expressing either RNA or dipeptides (DPRs) to model C9orf72 linked ALS in cells and mice.

Methods: Self-complementary AAV9 (scAAV9) viruses carrying GGGGCC repeats from 10 to 102 or the sequence encoding the DPRs were generated and tested *in vitro* before using in animals. *In vivo* experiments were performed by injecting these viral vectors via cisterna magna in postnatal day 1 (P1) wild type mice. The phenotype of these animals was evaluated by using behavioural tests, electromyography and histological analysis.

Results: Animals injected with AAV9-RAN102 repeats start to exhibit motor deficit at 11 months post viral delivery. However, mice injected with DPRs (GA34 and GA69) showed a decline in rotarod performance, as well as defects in CatWalk gait pattern starting from the first 4 months. Survival was also affected in 34-injected mice. Histological analysis revealed widespread of AAV9-mediated DPRs expression in the brain and the spinal cord. Together, these findings indicate that DPRs play a key role in the disease initiation. The generated models are being used to investigate mechanisms leading to neurodegeneration and ultimately for gene therapeutic development.

Conclusion: The current study revealed that the expression of DPRs alone could lead to C9orf72-linked pathology and motor dysfunction in mice. The generated models are useful tools to investigate mechanisms leading to neurodegeneration and therapy development.

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C68 DEGENERATION OF SEROTONIN NEURONS IS NECESSARY TO ELICIT SPASTICITY IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: serotonin, mouse models, spasticity

Serotonin neurons regulate a number of functions that are modified during disease progression, in particular motor neuron excitability or microglial activation. We previously demonstrated that brain serotonin neurons degenerate in ALS patients and mouse models (1,2), yet the consequences of this degeneration were unclear as were the serotonin receptors involved. The serotonin 2B receptor (5-HT2B), a serotonin receptor expressed in microglia, was upregulated in the spinal cord of three different transgenic mouse models of ALS. Ablation of 5-HT2B receptor in transgenic ALS mice expressing mutant SOD1 resulted in increased degeneration of microglia, as well as acceleration of disease progression (3). To gain more insights into the actual function of serotonergic neurons, we used a conditional mutant SOD1 transgenic mouse model to delete the ALS-causing transgene selectively in brainstem serotonin neurons in adult mice. This was sufficient to rescue the degeneration of these serotonin neurons and, most importantly, abolished the development of spasticity as assessed using electromyographic measurement of the long lasting reflex. Consistent with spasticity compensating the deterioration of motor function, rescuing serotonin neurons worsened motor function and accelerated the onset of paralysis. These findings demonstrate that the serotonergic system limits degeneration of spinal cord microglia, and is necessary to trigger at least part of the typical symptoms commonly attributed to UMN loss.

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C69 THE DISEASE MODIFYING EFFECTS OF EXERCISE AND SEDENTARY BEHAVIOUR IN A MOUSE MODEL OF MOTOR NEURON DISEASE

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Keywords: nerve stimulation, motor unit, exercise therapy

Background: Muscles with a higher proportion of fast twitch, quickly fatiguing motor units are the first to exhibit degenerative changes in the SOD1 transgenic mouse (1). Motor unit phenotype is altered by frequency and pattern of neuromuscular activity: endurance exercise reduces, while sedentary behaviour increases the proportion of fast fatiguing motor units (2). Interventions that change the

proportion of fast fatiguing motor units should alter disease progression if this is a causal relationship.

Objective: To determine if a causal relationship exists between changes in motor unit phenotype and disease progression in SOD1 mice.

Methods: Experiments were conducted in accordance with ARRIVE guidelines. A total of 203 mice were used in a cross-sectional, gender-balanced design with littermate matching of wild-type (WT) and transgenic mice (G93A SOD1 on B6SJL background). Primary outcome variables included motor unit number estimates (MUNE (3)), *in situ* contractile physiology and immunofluorescence analysis of myosin heavy chain type and size of muscle fibres in three muscles: tibialis anterior (TA), medial gastrocnemius (MG) and soleus (SOL). Researchers were blinded to genotype and treatment arm during analysis. Measurements were made on mice at 40, 60, 90 and 120 days of age for the time series component (N=102). (Age at end stage for male breeders was 137.5 ± 12.6 days, N=14.) The endurance exercise intervention used implanted stimulation of the sciatic nerve from 60 to 110 days of age (10 Hz, 8 hours/day, 5 days/week, N=56). The sedentary intervention used hind limb unloading from 40 to 60 days of age (24 h/day, N=26) with a subset of mice (N=19) that recovered from unloading for 30 days prior to measurement.

Results: The onset and progression of the disease confirmed the vulnerability of fast fatiguing motor units compared to slow endurance motor units. Stimulation reduced muscle fatigue with congruent increases in oxidative enzyme activity ($p<0.001$–0.015) and a modest fast-to-slow shift of muscle fibre type ($p<0.001$–0.024). While stimulation resulted in adaptation of muscle phenotype, there was no evidence for a change in muscle denervation (MUNE p=0.211 and 0.725). The sedentary intervention caused a slow-to-fast shift of muscle fibre type ($p<0.001$) and exacerbated disease progression ($p<0.01$). A period of recovery after unloading showed that deficits from sedentary behaviour persisted and onset of weakness was earlier.

Discussion: Reduced activity exacerbated disease progression but increased neuromuscular activity did not slow progression of the disease. Reduced activity following a diagnosis of motor neuron disease may be a risk factor influencing disease progression.

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C70 ROBUST BENEFICIAL EFFECTS OF A NON-COMPETITIVE AMPA RECEPTOR ANTAGONIST IN AN ALS MOUSE MODEL

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Keywords: AMPA receptors, calcium, model mice

Background: The candidate drugs that have been developed to treat ALS are largely based on studies in SOD1 transgenic ALS animal models, but virtually all of these drugs have proven ineffective. Therefore, a novel strategy for drug development that exploits evaluation markers that are closely linked with ALS pathogenesis would be valuable. Both TAR DNA-binding protein 43 (TDP-43) pathology and RNA editing failure at the glutamine/arginine (Q/R) site of GluA2, an α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor subunit, are characteristic etiology-linked molecular abnormalities that concomitantly occur in the motor neurons of the majority of patients with ALS. Adenosine deaminase acting on RNA 2 (ADAR2) specifically catalyzes RNA editing at the Q/R site of GluA2, and conditional ADAR2 knockout mice ($ADAR2^{flox/flox}$/VAChT-Cre.Fast; AR2) exhibit a progressive ALS phenotype with TDP-43 mislocalization through a Ca^{2+}-permeable AMPA receptor-mediated mechanism. Therefore, amelioration of the increased Ca^{2+} influx through the use of AMPA receptor antagonists may be a potential ALS therapy.

Objectives: Because amelioration of the exaggerated Ca^{2+} influx through the abnormal AMPA receptors could be a therapeutic target for sporadic ALS, and AR2 mice will be useful to evaluate the efficacy of treatment, we assessed the efficacy of systemic AMPA receptor antagonists on the AR2 mice.

Methods: Homozygous and heterozygous AR2 mice were used in this study. A non-competitive AMPA receptor antagonist perampanel (provided by Eisai Co., Ltd.) was administered daily to the mice via oral gavage for 14 days (either dose of 3.3, 6.6, 13.2 or 20 mg/kg to $AR2^{+/-}$ or $AR2^{-/-}$mice; n = 5–8 in each group) or for 90 days (at a dosage of 13.2 mg/kg/day for the first four days and then at 20 mg/kg/day for the remaining 86 days to $AR2^{-/-}$ mice; n=8). The age-matched control mice received oral gavage of the same volume of a 0.5% methyl cellulose solution for the same period. Effects on motor dysfunction were assessed by rotarod scores and grip power, and on ALS pathology were assessed by the number of spinal anterior horn cells and TDP-43 immunohistochemistry at the end of drug administration.

Results: Oral administration of perampanel to AR2 mice for 14 days effectively normalized TDP-43 pathology in motor neurons. All the mice completed the 90-day administration of perampanel and the progression of the ALS phenotype and the death of motor neurons were markedly ameliorated in the perampanel-treated AR2 mice. Moreover, the number of spinal motor neurons that exhibit nuclear localization of TDP-43 was markedly

increased in the perampanel-treated AR2$^{-/-}$ mice as compared to non-treated AR2$^{-/-}$ mice.

Conclusion: Given that perampanel has already been approved as an anti-epileptic drug, perampanel is a potential candidate ALS drug worthy of a clinical trial.

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Session 8A Clinical Genetics

C71 GENETIC PLEITROPY

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Keywords: genomics, precision medicine, pleitropy

In several clinical scenarios, the promise of "personalized medicine" has been translated into clinically actionable information. These advances are clearest in oncology, where the genetic profile of a given tumor strongly influences tumor behaviour, prognosis, and in some cases directs which therapies will be most beneficial. Because ALS risk and disease presentation are both highly genetic, additional discovery efforts are needed to uncover genomic variation and molecular biomarkers that will subclassify patients, forecast their disease course, and predict responses to emerging therapies. Most critically, the genetic findings must be considered comprehensively in order to understand how the perturbation of specific biological processes influence the risk of disease.

Here I review the remarkable progress in ALS genomics over the last decade and emphasize two key steps needed for the next phase of ALS genomics. First, genetic studies need to shift from archived samples with minimal or modest clinical data available to focus on ALS patients that are currently being followed clinically. This approach will allow rapid correlation of causal and candidate mutations with detailed clinical phenotypes, and will lay the foundation for genetically stratified trials. Second, genetic studies need to move beyond a focus on coding regions (e.g. exome sequencing) to interrogate the full genome through whole genome sequencing. I review developments in both areas.

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C72 GENETIC SCREENING OF 18,926 SAMPLES REVEALS NEW RISK ALLELES FOR FAMILIAL AND SPORADIC ALS

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Keywords: exome, genome, rare variant

Genetics is believed to be the major factor determining susceptibility to both familial (FALS) and sporadic (SALS) ALS. Despite the successful mapping of several major ALS risk genes, known disease variants cannot be identified in close to 30% of FALS and 90% of SALS cases. To identify new FALS risk genes, we applied gene based rare variant burden analyses to an exome resequencing dataset comprising 1022 index FALS cases and 7315 controls. In a novel approach, we conducted an optimization procedure where rare variant analyses were trained to best detect disease signatures from 10 established ALS genes (SOD1, TARDBP, FUS, VAPB, UBQLN2, OPTN, VCP, PFN1, TUBA4A, TBK1). Trained analyses were then applied exome-wide revealing a statistically significant association between FALS risk and loss of function (LOF) variants in the gene NEK1. Independently, we also conducted autozygosity mapping of SALS cases from an isolated community in the Netherlands, this revealed NEK1:p.R261H as a probable ALS risk factor. Replication analyses involving a total of 6172 SALS cases and 4417 controls confirmed significant disease associations for both NEK1 LOF variants and NEK1:p.R261H. Replication analyses were also performed for two additional genes identified by FALS analyses, both genes exhibited higher mutation rates in patients versus controls but sample sizes were not sufficient to evaluate statistical significance. In total, NEK1 risk factors are observed in ~3% of ALS cases making it one of the most important ALS genes discovered to date. The gene is known to function in several biological pathways of possible disease relevance including cilia formation, DNA damage response, microtubule stability, neuronal morphology and axonal polarity. Our work reveals NEK1 as a major ALS susceptibility gene and highlights candidate genes for future study. Our work also demonstrates the considerable benefits afforded ALS gene mapping efforts by large multi-centre collaborations.

Acknowledgements: This work represents the combined efforts of all members of (i) the FALS exome resequencing consortium and (ii) the Project MinE consortium.

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C73 PROJECT MINE GWAS: GENOME-WIDE ASSOCIATION ANALYSES IDENTIFY NEW RISK VARIANTS AND THE GENETIC ARCHITECTURE OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: GWAS, genetics, MinE

Background: Twin studies have estimated the heritability of ALS at around 65%, and therefore genetic risk factors play an important role in ALS susceptibility. Although an increasing number of genes involved in ALS risk have been identified, the majority of ALS heritability remains unexplained. Besides the *C9orf72* locus, GWAS have only identified two additional risk loci. Furthermore, the genetic architecture of ALS remains undefined.

Objectives: To find genetic risk loci and describe the genetic architecture of ALS.

Methods: Through Project MinE, we assembled a custom imputation reference panel from whole genome-sequenced ALS patients and matched controls (N = 1861). Using this panel, 8,697,640 variants were imputed in 12,577 ALS cases and 23,475 controls. These loci were tested for an association with ALS risk applying a linear mixed model. New loci were subsequently replicated in 2579 cases and 2767 controls in an independent replication cohort. To describe the genetic architecture we applied polygenic risk scoring and estimated the SNP-based heritability of ALS using three different methods (GCTA-REML, LD-score regression and Haseman-Elston regression). We partitioned this heritability by chromosome and allele frequency.

Results: The custom, ALS-enriched, imputation panel improved imputation of low frequency variants compared to publicly available panels such as the 1000 Genomes Project. In total we found six robustly associated ($p < 5.0 \times 10^{-8}$) loci: *C9orf72, UNC13A, SARM1, C21orf2, MOBP* and *SCFD1*. The *TBK1* locus approximated genome-wide significance ($p = 6.6 \times 10^{-8}$). The SNP in *C21orf2* represents a low frequency (MAF = 1.6%) non-synonymous variant. We fine-mapped the *C21orf2* locus, by showing an increased burden of rare non-synonymous variants in *C21orf2,* in an independent sample of 2562 cases and 1138 controls also sequenced as a part of Project MinE. A significant proportion of the phenotypic variance could be explained by polygenic risk scores (Nagelkerke r^2 = 0.44%, $p = 2.7 \times 10^{-10}$) establishing ALS as a polygenic trait. The heritability explained by all SNPs was estimated at 8.5%. In contrast to other polygenic traits, the majority of the heritability in ALS was explained by low-frequency variants.

Discussion and conclusion: We have identified a key role for rare variation in ALS, discovered SNPs in new complex loci and established *C21orf2* as a novel ALS risk gene. Our study informs future study design in ALS genetics, promoting the combination of larger sample sizes with full genome coverage to fine-map new loci, identify rare causal variants and thereby elucidate the biology of ALS.

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C74 SHARED NOVEL VARIANT ANALYSIS IDENTIFIES NOVEL GENES IN FAMILIAL ALS FROM WHOLE EXOME SEQUENCING

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Keywords: exome sequencing, rna-binding, novel gene

Background: Whole-exome sequencing (WES) has proven to be an extremely successful technology for the discovery of novel genes and mutations in Mendelian disorders. We have sequenced all 270 available DNA samples from Familial ALS patients collected by King's College Hospital, Guy's Hospital and the UK MND DNA Bank (1), and smaller cohorts donated by other collaborators. Combining this data with cohorts sequenced by other collaborators resulted in a total cohort size of 1008 patients which, after cases with a known pathogenic mutation had been excluded, included 750 index cases and 68 of their affected relatives.

Objectives: The objective of this study is to discover novel candidate genes, and novel mutations within those genes, which are causative of familial ALS.

Methods: Next generation sequence reads were aligned to the hg19 reference genome via BWA, variants called with Samtools Mpileup, annotated with Annovar plus custom perl code and quality filtered, giving over 1 million unique exonic or splice site changes. Variants were further filtered to include only those that were novel (defined as being absent from ExAC, EVS, UK10K, 1000genomes and 670 local control exomes, n=>70,000) and found in 3 or more FALS probands.

Results: Eight novel variants shared between 3 or more FALS probands were identified. Six of these have been previously published as causative for ALS in the genes SOD1, FUS and TARDBP, and a seventh was presented at the 2015 International Symposium on ALS/MND (2). A Proline to Leucine amino acid change was found in 3

unrelated probands in a gene which has not previously been linked to ALS. Close examination of a low-coverage exon of this gene, coupled with Sanger re-sequencing, revealed a second novel Proline to Leucine variant in 11 FALS probands, 2 affected relatives and 2 SALS cases, and a novel change to a Glutamine residue at the same location in two other SALS. ExAC also has severely reduced coverage in this exon, but after Sanger sequencing of 2,150 population-matched controls the three variants remain novel. This gene has not previously been well studied but is expressed primarily in the CNS and skeletal muscle, contains an RNA-binding domain and undergoes complex alternate splicing. Work is ongoing to validate isoform-specific antibodies, determine the most disease-relevant transcripts, and to decipher the mechanism of pathogenicity of the ALS-specific mutations in cellular and model systems.

Discussion and conclusion: Few large pedigrees remain in Familial ALS from which linkage can successfully be performed. Burden tests require large numbers of control exomes, which are prohibitively expensive to produce. The discovery of novel genes in FALS benefits greatly from combining multiple cohorts in order to achieve sufficient power, and one should exercise caution when rejecting variants located in poorly covered regions.

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Session 8B Technology and ALS

C75 THE POWER OF SHARING DATA: 10 YEARS EXPERIENCE WITH PATIENTSLIKEME

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Keywords: disease progression, natural history, social support

Background: People with ALS (PALS) and caregivers have been using the Internet since the early 1990's to share information and support through email listservs and bulletin boards. A decade ago a family affected by ALS launched PatientsLikeMe.com (PLM), a patient-powered research network that allowed members to share data, not just stories.

Objective: To describe the international population of PLM members, their self-reported ALSFRS-R trajectories, symptoms over time, evaluations of key treatments, benefits reported by interacting with their peers, and contributions to the research literature.

Methods: Descriptive analysis of users including age at onset, diagnostic latency, site of onset, and familial status (with intra-country comparisons where available). A survey of N=115 patients with ALS asked to report their healthcare utilization, Patient Activation Measure (PAM) score, strength of peer network before/after PLM, and benefits arising from use of PLM. Literature review of scientific contributions to the literature and public advocacy.

Results: Since launch over 8000 patients with ALS have registered. Most members are male (57%), reported a limb onset in their fifties, had a diagnostic latency of around one year, and live in the United States (73% of patients reporting). Family history of ALS was reported by 10% of members reporting. Around half of the patients ($N \sim 4000$) have completed symptom severity scores for 10 key symptoms; fatigue (55% report moderate or severe), stiffness/spasticity (46%), fasciculations (45%), excess saliva (30%), pain (28%), anxious mood (25%), insomnia (25%), emotional lability (24%), constipation (24%), depressed mood (23%), and excessive yawning (22%). The number of moderate/severe symptoms experienced increases by approximately 1 symptom every 2 years during the first 5 years following onset. Many of the treatments reported as most effective for symptom management were not pharmaceutical but included wheelchairs, ventilators, massage, or over the counter medication. Many of the pharmaceutical treatments reported as helpful were being used off-label, such as the antidepressant amitriptyline for excessive saliva. There was a bimodal distribution on the PAM, with 24% of patients at the lowest level of activation (1, disengaged), 16% level 2 (becoming aware), 44% level 3 (taking action), and 17% level 4 (maintaining behaviours). Benefits of PLM reported included a better understanding of what might help members live better with their condition (77%), available treatments (71%) and how their condition might affect them (71%). As a result of PLM, 55% reported better interactions with their healthcare professionals, 49% better symptom management, 25% started a new treatment, and 18% were more adherent. Patient data contributed to 10 peer-reviewed studies and 27 ALSUntangled investigations.

Discussion: Despite shortcomings such as bias, the opportunities for research and peer support appear promising.

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C76 TELEHEALTH IN MOTOR NEURONE DISEASE. A MIXED METHODS, RANDOMISED CONTROLLED, PILOT STUDY OF THE USE OF THE TIM TELEHEALTH SYSTEM TO DELIVER HIGHLY SPECIALISED CARE IN MOTOR NEURONE DISEASE, AT A DISTANCE

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Keywords: telemedicine, multidisciplinary care, quality of life

Background: Specialist multidisciplinary care for patients with motor neurone disease (pwMND) is associated with increased survival. We have developed the TiM telehealth system (Telehealth in motor neurone disease), designed to improve access to specialist care. Patients and carers use the TiM "app" on a tablet computer to record details of their clinical condition, weight and balance score. The MND care team can monitor the results remotely using a bespoke clinical portal.

Objectives: To assess the feasibility and acceptability of using the TiM telehealth system in clinical practice; to pilot the methods and assess the feasibility of conducting a definitive trial.

Methods: pwMND and their primary carer were randomized to receive either usual care or the TiM telehealth system plus usual care for 6–18 months. Semi-structured interviews were conducted at baseline (controls), one and six months (intervention) to explore participants' experiences of MND care, attitudes towards technology and their experiences of being in the trial and using the TiM system. Clinicians were interviewed to explore their experiences of the TiM system. Thematic analysis was undertaken and results triangulated with data including adherence to the TiM system, clinical, quality of life and satisfaction outcomes. Early feedback resulted in improvements to the TiM system being implemented and evaluated during the study.

Results: We recruited 40 pwMND (30% female) aged 39-78 years, plus 37 informal carers (76% female). 20 pwMND and 18 carers used the TiM system. Follow-up finished in May 2016 and detailed results will be available later in the year. Qualitative data suggest that the TiM system was easy to use, even by those with severe disability or no technology experience. Patients in the early stages of the disease reported that the TiM provided reassurance and self-awareness of the disease. Patients with more advanced disease perceived value in improving communication and problem solving. Challenges included how to respond to the large volume of clinical data collected by the system and the differing expectations and priorities of participants, MND nurses and physicians. Many patients felt it would be acceptable to use the TiM as an alternative to clinic appointments, particularly in the early stages of disease, or if they had no active problems. They also felt the TiM could help maintain contact if they became too unwell to travel.

Discussion and conclusion: The TiM telehealth system is an acceptable and feasible way to improve access to specialist care. Further work will develop the TiM system for use in different clinical services and evaluate its impact on clinical and health economic outcomes.

Acknowledgements: The TiM was developed in collaboration with Mylan. The trial was funded by a National Institute of Health Research Doctoral Research Fellowship, Mylan and the Motor Neurone Disease Association.

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C77 OPTIMIZING CARE THROUGH TELEMONITORING IN VENTILATED PATIENTS WITH MOTOR NEURONE DISEASE: A PILOT STUDY

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Keywords: non-invasive ventilation, telemonitoring/telemonitoring, symptom management

Background: We are interested in optimizing care for people with motor neurone disease (MND) who are on non-invasive ventilation (NIV) and whether technology can contribute. We have previously developed a respiratory question set for telemonitoring to complement the monitoring of continuous overnight oximetry and patient ventilator interactions (PVI).

Objectives: We wished to examine whether proactive management was feasible, determine the work generated for the clinical team and the impact on the maintenance of ventilation.

Methods: Ten ventilated patients (male=6; mean age=61yrs; median illness duration=14 months; median NIV usage=11 months) were recruited and followed for 6 months. Participants used a telemonitoring device (Careportal®) to complete the respiratory questions; nocturnal pulse oximetry and PVI were also assessed weekly. Although monitored, patients were told to contact their general practitioner (GP) for emergencies. Revised ALS functional rating scale (ALSFRS-R) and Hospital Anxiety and Depression Scale (HADS) were completed three-monthly. Non-parametric tests were used to identify changes in oxygen saturation levels (SpO2), PVI, ALSFRS-R, and HADS over the trial period.

Results: In total, 118 telemonitoring question alerts led to 40 interventions in 8 patients (equipment provision=16, treatment adjustment=15, direct reviews=8, referral=1), these were needed despite 5 having had recent routine outpatient review. Twenty-four interventions were triggered by the answers to the respiratory questions, 8 were requested by patients through Careportal®, 5 had pressure adjustments to correct suboptimal nocturnal oximetry and 3 were follow-up interventions. Three patients made unscheduled GP contacts and one of them was hospitalised with pneumonia, despite being on antibiotics. Amongst PVI data, minute ventilation (MV) was maintained; baseline mean=6.32 l/min (SD=1.81) and endpoint mean=7.2 l/min (SD=2.64). Inspiratory positive airway pressure (IPAP) (7 patients had alterations) and NIV usage time changed significantly; baseline IPAP cmH_2O mean=16.97 (SD=4.2) and end point mean=20.53 (SD= 5.46; p=0.004); baseline NIV usage mean=8.55hrs (SD= 3.32) and endpoint mean=11.45hrs (SD=3.55; p=0.006). ALSFRS-R scores deteriorated: baseline mean=22.9 (SD=10.9) and end point mean =18.8 (SD=10.19; p=0.008). HADS scores for anxiety did not change from baseline (mean=4.5; SD=3.37) to end point (mean=3.9; SD=3.07) or for depression from baseline (mean=6.3; SD=3.47) to endpoint (mean=8.3; SD=4.85).

Discussion and conclusions: We found weekly telemonitoring to be feasible. Optimization of SpO_2 levels was enabled and ventilation was maintained. The bespoke questions triggered 60% of the interventions, highlighting the importance of symptom review in addition to physiological or PVI measurements. Maintained HADS suggest no adverse effect of telemonitoring, this and whether it is perceived as useful is being explored through qualitative work. This approach may replace some traditional reviews, linking the patient effectively to the care team. Further study should be conducted to examine its impact on the holistic management of this patient group including health economics and outcomes.

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C78 E-LEARNING LEARNING FOR ALS HEALTH CARE PROVIDERS

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Keywords: nurse specialist, ALS-careteam, education

Background: Many of these health providers lack sufficient experience with and knowledge of ALS. The ALS Centre Netherlands has therefore developed courses consisting of both e-courses and face-to-face training.

Objectives: To enhance health care professionals' knowledge and skills concerning swallowing problems and screening for the need of psychosocial counselling for ALS patients.

Program description: The e-courses are designed to elevate professional knowledge. Additional face-to-face training sessions aim to discuss problems as experienced, e.g. in the end of life phase. Four e-courses are available. The first is the free e-course "Introduction to ALS", which is obligatory before following any face-to-face training provided by the ALS Center Netherlands. The other e-courses have a small registration fee and focus on, (i) swallowing problems, (ii) recognizing unrealistic illness perceptions, cognitive and behavioural symptoms and emotional deregulation, (iv) motivational interviewing techniques to let patients and informal care givers reflect on unrealistic illness perceptions. More e-courses are currently being developed. The total duration of an e-course is on average one hour. The e-courses contain web lectures, text, assignments and multiple choice self-tests with feedback.

Method: The e-course "Introduction to ALS" has been followed by 464 persons during the first six months that this e-course was online. Health care practitioners who have followed this e-course include nurses, physiotherapists, speech therapists, occupational therapists, dieticians and social workers. The course was evaluated positively, with 98% of the responders (N=266) saying that they would recommend this e-course to their colleagues. The usability, content and usefulness were evaluated positively with average scores >4.0 on a 1–5 scale. Participants reported positive experiences with the flexibility of following an e-course anytime anywhere. Participants agreed with statements that they had reached the learning objectives, such as knowing the symptoms, diagnostic process, average ALS disease progression and treatment of symptoms (average scores >4.0 on a 1-5 scale). The e-course "Swallowing" was followed by 39 practitioners in the first 4 months of being online and the e-course "Recognizing unrealistic illness perceptions" has been followed by 27 care providers so far.

Clinical Outcomes: More evaluation results will be available from November 2016.

Recommendations to the Field: Use blended learning, combining the flexibility of e-learning with the in-depth discussions of face-to-face training; involve educational experts to develop the best mix of learning activities.

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C79 AUGMENTATIVE AND ALTERNATIVE COMMUNICATION FOR LOCKED-IN STATE PATIENT

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Keywords: Augmentative Alternative Communication, Locked-in Syndrome, Brain-Computer Interface

Background: Locked-in Syndrome (LIS) is a severe motor disability in which patients are conscious and alert but unable to use their muscles and therefore cannot communicate. Causes include neurodegenerative diseases in particular Amyotrophic Lateral Sclerosis. Some spared movement can be present and visible or measurable by electromyography (1). In Completely Locked-in Syndrome (CLIS) patients lose any control of the muscular response. Braincontrol is a core solution of a bio-feedback framework based on artificial intelligence techniques for human-computer interaction that allows these patients to control assistive technology and to widen the options of augmentative and alternative communication (AAC) (2).

Objectives: The objective of this study is to validate Braincontrol as a multi-channel AAC device. In order to adapt the system to different degrees of disabilities, the device is structured on multiple interaction methods on the basis of the needs of the patients.

Material and methods: The Braincontrol AAC is a platform that allows people suffering from neuromuscular degenerative diseases to overcome physical and communicative disabilities enabling several methods of interaction: Brain-computer interface (BCI), a technology that converts brain electrical signals into commands able to control external devices without need of any voluntary movement, focusing on CLIS and patient unable to give any kind of feedback (3); Eye Tracking (ET) Standard, an interaction channel used by fixing the gaze on the interface point of interest; ET-Easy, used in case of limited mobility of the pupil and no need for calibration; Motion detection (MD), which uses inertial sensors, infrared and/or webcam tablet PC to map and detect residual volunteers movements; Touch, designed to be used in presence of reduced mobility of the upper limbs. This study, conducted from 2012 to 2016, involved 60 patients with communication and motility disorder. Of these, 56 out 60 used BCI technology, 3 used ET and 1 used MD sensor.

Each patient has been classified with an initial functional evaluation and trained for 4 session with the appropriate interaction channel.

Results: 58 out of 60 patients reached a good communication level with a percentage of 96.7%. The 2 patients who have not regained the capacity to communicate were in the condition similar to the CLIS in which cognitive and psychological status information were unknown.

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Session 8C Evolving Biomarkers

C80 TISSUE-ENHANCED PROTEOMIC ANALYSIS OF PLASMA SAMPLES REVEALS NEW MECHANISTIC BIOMARKER CANDIDATES FOR THE STRATIFICATION OF AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Keywords: biomarkers, proteomics, mass spectrometry

Background: Early diagnosis and patient stratification in ALS based on disease sub-type and progression rate requires the ability to measure objectively the molecular changes underlying the development of disease. The simultaneous analysis of diseased tissues and biological fluids using a powerful and revolutionary proteomic technique called TMTcalibrator™, enhances detection of low-abundant, tissue-derived proteins that reflect the central disease processes in the periphery. These are powerful biomarkers that should lead to a better clinical stratification of ALS patients.

Objectives: To co-analyse the plasma and peripheral blood mononuclear cell proteomes from patients with different forms and rates of progression of ALS and equivalent mouse models to identify co-expressed proteins, which are differentially regulated at different stages of disease and may serve as useful stratification biomarkers.

Methods: TMTcalibrator™ was employed to enhance detection of disease-associated plasma proteins. PBMC digests were used as the calibrator, spiked in a 1.75:1 excess to plasma samples from patients stratified according to progression rate and disease stage. Six patients with a slow progression rate (monthly slope of ALSRS-R from disease onset to last visit or progression rate to last visit, PRL <0.5) and 6 patients with a fast progression rate (PRL >1) were studied. For each patient, two plasma samples were included in the study from an early (ALSRS-R (/48) >40) and a late disease stage (ALSRS-R (/48) <30). An equivalent study was performed in PBMCS/plasma from mouse models reproducing a similar fast and slow disease progression.

Results: We have identified approximately 4500 unique proteins in each data set. We first analysed data by time point to identify 95 differentially regulated proteins between slow and fast progressors in early samples, and 107 significantly regulated proteins in late stage samples. When considering slow and fast progressing patients separately, 625 proteins (259 unique, 366 shared) were differentially expressed between early and late samples for the slow progressing patients and 525 (159 unique, 366 shared) for the fast progressing patients. Functional annotation was performed to extract relevant biological interpretation in the context of known mechanisms of ALS. *In silico* comparisons of these results with those obtained from the mouse models have also been performed to highlight those proteins that are detected as regulated in both species.

Discussion and conclusions: This is the deepest proteomic profiling yet undertaken on plasma from ALS patients. The TMTcalibrator™ technique has provided a unique opportunity to study the interface between cellular and fluid components of blood in unravelling of ALS pathology. The parallel analysis of mouse models recapitulating a similar heterogeneity of the disease will provide a formidable working platform with translational potential and a dataset to support researchers working in ALS.

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C81 ELECTRICAL IMPEDANCE MYOGRAPHY FOR EARLY DIAGNOSIS AND ASSESSMENT OF ALS PROGRESSION: RESULTS OF A MULTICENTER CLINICAL TRIAL

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Keywords: biomarker, progression, clinical trials

Background: In order to improve efficiency of clinical trials in ALS and more rapidly identify effective therapies, improved diagnostics and biomarkers of disease progression are needed. One potential technology for this purpose is electrical impedance myography (EIM). In EIM, a weak electrical current is applied over a localized area of muscle and the consequent surface voltages are measured. Alterations in the condition of the muscle produce changes in these voltages that can be used to assist in diagnosis and to track disease progression over time. Previous clinical studies showing sensitivity to progression in ALS have used off-the-shelf impedance systems not intended for muscle application (1,2); whether a dedicated muscle system can prove more effective is unknown.

Objectives: To assess a dedicated multifrequency EIM system, the EIM 1103 from Skulpt, Inc, to assist with early diagnosis and to track disease progression over time in ALS.

Methods: A total of 47 ALS patients (age range 42–78, 31 men/16 women), 30 healthy controls (age range 36–68, 17 men/13 women), and 30 ALS "mimics", including patients with weakness that could be mistaken for ALS, (age range 39-81, 18 men/12 women) were recruited across 6 study sites. All subjects underwent multifrequency EIM of multiple muscles, including bilateral deltoids, biceps brachii, forearm extensors, vastus lateralis, tibialis anterior, and medial gastrocnemius as well the thoracic paraspinal muscles. ALSFRS, handheld dynamometry, and SVC were also performed. For group classification, analyses will include the development of receiver operating characteristic curves and multivariate clustering. For assessment of progression a mixed random slopes model will be utilized; sample size estimations for hypothetical clinical trials will also be calculated.

Results and discussion: Preliminary analysis shows that 50 kHz phase successfully differentiated ALS patients from healthy subjects ($p < 0.0001$) but not from ALS mimics ($p = 0.13$). However, the 100/300 kHz phase ratio did so successfully for both groups ($p < 0.0001$ compared to controls and $p = 0.027$ compared to mimics). In terms of tracking disease progression, mean 50 kHz phase values showed a $0.18 \pm 0.23°$/month mean decrease across all muscles, giving a coefficient of variation in the rate of decline of 1.28. The most rapidly declining muscle however showed a $0.70 \pm 0.46°$/month decrease in phase, giving a coefficient of variation in the rate of decline of 0.64; the 100/300 kHz ratio performed still better, reaching a value of just 0.58.

Conclusions: Preliminary analysis confirms EIM's ability to differentiate ALS patients from healthy controls and also from disease mimics. Consistent with a previous study (2), EIM focused on the most rapidly progressing muscle appears to be very sensitive to disease progression. A full analysis using the planned statistical methods described above will be presented at the meeting.

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C82 MEG CORTICO-MUSCULAR COHERENCE TO ASSESS CORTICOSPINAL TRACT INTEGRITY IN ALS

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Keywords: biomarker, neurophysiology, magnetoencephalography

Background: Histopathology and structural MRI have demonstrated the consistent vulnerability of the corticospinal tract (CST) in ALS pathogenesis. Assessing integrity of the CST using transcranial magnetic stimulation relies upon non-physiological manipulation of cortical activity, whereas cortico-muscular coherence (CMC) is a reliably observed dynamic index of healthy sensorimotor function.

Objectives: To assess CST integrity by measuring CMC using magnetoencephalography (MEG).

Methods: Fifteen ALS patients and 11 healthy controls were studied. Participants lightly gripped a fibre-optic force measurement response bar in each hand prior to executing a visually cued, temporally prepared, unilateral forceful grip response. CMC was exclusively calculated during the light grip hold. After coregistration to T1 structural MRI, MEG data were beamformed to extract a neural activity timecourse from each motor cortex. Contralateral connectivity to rectified surface EMG signals recorded from forearm flexors was calculated using two complementary methods: phase coherence and Hilbert amplitude correlations. CMC group differences were calculated using cluster permutation statistics across the frequency range.

Results: Healthy controls demonstrated a clear peak in CMC around 20 Hz (beta-band). In contrast the ALS patient group lacked a distinct peak at any frequency, with phase coherence reduced around 25Hz (p=0.02), and amplitude correlation reduced around 20Hz (p=0.01).

Discussions and conclusions: Abnormal CMC is a feature of ALS that reflects impaired transmission of sensorimotor neural signals from cortex to the peripheral musculature due to CST damage. CMC is readily quantifiable and as a future biomarker of CST integrity may add diagnostic value, and have value as an early indicator of therapeutic benefit.

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C83 AUDITORY MISMATCH NEGATIVITY IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: electroencephalography (EEG), mismatch negativity, cognition

Background: Amyotrophic Lateral Sclerosis (ALS) is recognized as the neurodegeneration of motor neurons, which additionally affects non-motor domains such as cognition. Quantifying this cognitive deficit in ALS patients using electrophysiological measures remains elusive. Auditory mismatch negativity (MMN) is a neurophysiological response elicited by the unexpected change in a series of repeated stimuli, which has been used to quantify cognitive impairment in different neurological disorders.

Objectives: This study aims to examine potential reductions in amplitude or increases in delay in the MMN waveforms of ALS patients, which would indicate deterioration in networks underlying cognition.

Method: High density (128 channel) EEG data was recorded from 90 ALS patients (68 spinal, 15 bulbar, and 7 cognitive onset) and 19 controls. Subjects were presented with standard auditory tones interleaved with 10% frequency-deviant (oddball) tones in 3 recording blocks, each lasting 8 minutes. The significance of between-group differences of the MMN waveforms were tested using the Mann–Whitney U test (α=0.05). The average delay of MMN, which was quantified as the time corresponding to the center of area underneath the MMN curve, was compared for patients and controls.

Results: The MMN waveforms were significantly attenuated (p<0.05) in the ALS group between 100 and 170ms post-stimulus in fronto-cortical regions (between FCz and Fz electrodes). The average delay (center of area) of the MMN waveform was significantly increased (p=0.0042, β = 0.05–0.17) for ALS patients. This finding correlated with neuropsychological assessment of executive impairment.

Discussion: The slowing of MMN response is a neurophysiological confirmation for previously reported cognitive dysfunction in ALS. This may be due to disruptions in brain networks involved in cognitive processes, including the salience network, in ALS. Detection of a delayed MMN response may serve as a potential prognostic tool or biomarker for the tracking of cognitive deficit in ALS patients.

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C84 GLIAL ACTIVATION MEASURED BY [11C]-PBR28 PET CORRELATES WITH 1H-MRS BRAIN METABOLITES IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: PBR28, MRS, neuroinflammation

Objectives: To evaluate the relationship between glial activation assessed by [11C]-PBR28 positron emission tomography (PET), and neuronal integrity and gliosis/neuroinflammation measured by proton magnetic resonance spectroscopy (1H-MRS) in people with amyotrophic lateral sclerosis (ALS).

Background: 1H-MRS and PET were employed to study *in vivo* molecular changes in the brain of people with ALS. Prior MRS studies show alterations in brain metabolites in the motor cortices and brain stem in people with ALS (1) and previous PET studies provided evidence of increased glial activation in ALS using the radiotracer [11C]-PBR28 PET (2). Combining multiple neuroimaging modalities is of importance to unraveling ALS disease mechanisms. To our knowledge, this is the first study to evaluate the relationship between glial activation, measured by [11C]-PBR28 PET, and brain metabolites assessed by MRS.

Methods: A total of 28 participants with either limb onset ALS (N=16), bulbar onset (N=7), or primary lateral sclerosis (N=5) were included in this study. The participants underwent brain imaging using MR/PET imaging with the radiotracer [11C]-PBR28 and simultaneous MRS. MRS data acquired from multiple single-voxels ($2 \times 2 \times 2$ cm^3) located in five positions in the brain, including the left motor cortex (21 subjects), right motor cortex (19 subjects), brain stem (8 subjects), frontal cortex (8 subjects), and parietal cortex (13 subjects). LC model was used to analyze MRS data and to measure brain metabolite ratios pertaining to neuronal viability (N-acetylaspartate/creatine, NAA/Cr) and glial activation/inflammation (myo-Inositol, mI/Cr). Freesurfer's tools were used to create 3D volumes that mimic the MRS Voxels. These volumes were moved and co-registered to the anatomical images, then [11C]-PBR28 uptake was calculated for every brain parcellate within these volumes. Pearson correlations were conducted to study the relationship between [11C]-PBR28 uptake and brain metabolite ratios within each voxel.

Results: Pearson correlation coefficient revealed an inverse relationship between glial activation, represented by [11C]-PBR28 uptake and NAA/Cr in the left motor

cortex ($r = -0.51$; $p = 0.01$) but not in other brain regions. In addition, a positive relationship was found between [11C]-PBR28 uptake and mI/Cr, both markers of gliosis/inflammation, in the right motor cortex ($r = 0.56$; $p = 0.01$) and brain stem ($r = 0.78$; $p = 0.02$). No correlations were detected in the frontal or parietal cortices.

Conclusion: Glial activation (PBR28 uptake) correlates with neuronal damage (↓NAA) and gliosis/neuroinflammation (mI) in brain regions affected in ALS.

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Session 9A Neuron–Glia Interactions

C85 ASTROCYTE TOXICITY IN MODELS OF IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: astrocyte, neurodegeneration, APP

Consistent with a non-cell-autonomous role in the pathogenesis of amyotrophic lateral sclerosis (ALS) are our demonstrations that wild-type primary and embryonic stem cell-derived motor neurons (ES-MNs) selectively degenerate when cultured in the presence of mutant SOD1 (mSOD1)-expressing astrocytes (1). We further found that this death phenotype is not restricted to mouse cells or to familial form of ALS, since astrocytes grown from post-mortem samples of sporadic ALS patients killed human ES-MNs (2).

Data from both our familial and sporadic ALS models revealed that MNs degeneration is caused by an astrocyte-mediated toxic activity (not a loss of beneficial effect). A series of biochemical experiments demonstrated that the toxic factor(s) is/are proteinaceous in nature and of $\sim$20 kDa. The silencing and immunodepletion of SOD1 from our experiments demonstrate that mSOD1 was unlikely to be the culprit. Conversely, the combination of unbiased proteomics and genomics studies points toward APP to contribute to astrocyte-mediated toxicity and DR6 to transduce the death signal in MNs. Further investigations enabled us to show that APP is deleterious via the production of a beta-soluble fragment that includes APP E1 domain. Yet, from *in silico* analysis, we do not believe that this APP fragment behaves as a bona fide ligand of DR6 but rather as an interactor promoting DR6 dimerization and ligand-independent activation.

Our findings support an involvement of non-cell autonomous mechanisms in ALS pathogenesis via a APP/DR6 mechanism. This process may have to be fully characterized to acquire a better understanding of the neurobiology of ALS and devise effectively therapies.

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C86 EARLY STAGE MOTOR NEURONS NEUROPROTECTION VIA ASTROCYTES RESTRICTED NF-KB ACTIVATION

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Keywords: NF-kB, astrocytes, neuroinflammation,

Background: Non-cell-autonomous mechanisms involving glial cells are critical for the unfolding of the pathogenic cascade in SOD1 G93A transgenic mice. However, the role of astrocytes and microglia (and the ongoing immune response) may change over time in disease progression, so far it has remained open on whether and when astrocyte-driven inflammation may be protective or detrimental.

Objectives: We have used a genetic strategy based on conditional activation of the IKK/NF-kB signaling pathway to achieve spatio-temporal control of astrocytic-orchestrated neuroinflammation and explore its contribution at different time points during disease progression.

Methods: We generated a triple transgenic mouse in which astrocyte-specific NF-kB activation is achieved by doxycycline-inducible expression of a constitutively active allele of IKK2 in the SOD1 G93A mouse model; the activation of NF-kB was restricted to astrocytes using a GFAP-promoter-driven tTA gene. After induction of the IKK2-CA transgene, we have monitored motor performance, disease marker and neuroinflammatory phenotype.

Results: Compared to mSOD1 littermates, the GFAP.tTA/tetO.IKK2-Ca/mSOD1 G93A triple transgenic mice (IKK2 induced from P20) displayed a prolonged time to weight inflection point (onset) but an accelerated progression phase (from onset to exitus), leading to an unchanged lifespan. Likewise, triple-tg mice spent longer time in milder clinical stages (lower clinical score) but reached the same level of disability at later time points. Therefore, NF-kB activation in astrocytes causes significant but biphasic effects on motor performance during the disease course. In early stages, when beneficial effects on motor performance are detected, we observed a significant decrease in MN disease markers (such as misfolded SOD1 burden, LC3A and p62 buildup) in correspondence of a massive rise in microglial density, amoeboid morphology and of a CD45 + microglial

subpopulation appearance together with a significant CD4 + cells infiltration. At this stage, STAT-6 pathway is upregulated in astrocytes as well as in lymphocytes suggesting a IL-4/IL-13 autocrine-paracrine mechanism. However, starting from P70 in the triple-tg mouse microglia assumed a CD68 + phenotype with a senescent morphology, whereas MN marker levels were equal to the mSOD1 mice, suggesting a loss of the beneficial effect driven by astrocytic IKK2/NF-kB activation. This effect keeps going on at P90 as well, accompanied with a degenerating microglia.

Conclusion: Taken together our data demonstrated a biphasic role of inflammation in ALS pathogenic cascade, where astrocyte-orchestrated amplification of the inflammatory response is beneficial at early stages, but it turns to detrimental at later time points. The elucidation of the mediators involved in the proliferation processes and lymphocytic function during the beneficial phase, may offer new entry points for translational therapeutic interventions.

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C87 NEURONAL PATHOPHYSIOLOGY IN A HUMAN IPSC MODEL OF ALS INVOLVES INTERPLAY BETWEEN ASTROCYTES AND MOTOR NEURONS

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Keywords: excitability, ionic currents, C9orf72

Background: Changes in the input–output relationship, or excitability, of motor neurons (MNs) represent one of the earliest pathological features reported in animal models of ALS. Recent work has shown that MN excitability also changes in human induced pluripotent stem cell (iPSC) models of ALS (1). Initially, iPSC-derived MNs harbouring ALS-related mutations display hyperexcitability, characterised by an increase in action potential firing. This is later followed by hypoexcitability, or a decline in firing capability, along with reduced ionic currents (1). Prior work suggests that non-cell autonomous processes involving astrocytes might contribute to such pathophysiological changes in MNs (2).

Objectives: This study was designed to investigate the potential impact of ALS-affected astrocytes on MN excitability and the ionic currents of MNs.

Methods: We used cultures with different compositions of MNs and astrocytes derived from iPSCs of ALS patients and healthy control individuals. These included co-cultures of astrocytes and MNs or enriched cultures of MNs with minimal astrocytes. The functional activity of MNs was investigated using whole-cell patch-clamp electrophysiological recording techniques in both current and voltage clamp modes.

Results: In co-culture experiments, we found no overt effects of astrocytes harbouring C9orf72 mutations on the viability of control MNs. We also found no evidence for hyperexcitability of control MNs in the presence of ALS-affected astrocytes. However, astrocytes harbouring C9orf72 mutations induced hypoexcitability in control MNs, which was associated with loss of voltage-activated sodium and potassium currents. Conversely, in neuron-enriched cultures with minimal presence of astrocytes we observed only very mild hypoexcitability with no change in voltage-activated currents.

Discussion and conclusions: Our data reveal involvement of astrocytes, via non-cell autonomous disease mechanisms, in MN pathophysiology in ALS. The expression of mutant C9orf72 in iPSC-derived astrocytes is sufficient to induce loss of output and reduced ionic currents in control iPSC-derived MNs. In contrast, without the influence of sufficient numbers of ALS-affected astrocytes, MNs expressing mutant C9orf72 show little or no evidence of pathophysiological changes. Together these findings highlight astrocyte-neuron signalling as an important potential target for novel ALS therapeutics.

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C88 PREDICTING NOVEL RELATIONSHIP OF THE CAUDAL DISTRIBUTION OF LOWER MOTONEURONS IN RELATION TO THE REGION OF CLINICAL ONSET TO THEIR CONNECTED GLIA IN SPORADIC ALS PATIENTS

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Keywords: sporadic ALS, neuroinflammation, neurodegeneration

Background: Recent observations on the location and spread of motoneuron degeneration in sporadic (sALS) patients suggest neurodegeneration is radially graded (1), and regions of minimal motoneuron loss are remote from site of onset. Increasing evidence suggests that neuroinflammation mediated by activated glia and infiltrated

immune cells are involved in progression (2). However, the mechanism by which activated glia induce disturbance of lower motoneuron loss is unclear.

Objectives: To determine the relationship of glia to the distribution of lower motoneurons in relation to the region of clinical onset in sALS.

Methods: We applied novel bioinformatics analysis methods (3) to transcriptional profiles established by exon microarrays of motoneurons isolated by laser capture microdissection from 12 sALS and 10 control patients (4) to identify the relationship of lower motoneurons and glia.

Results: We identified 5 clusters of co-expressed genes ("interactomes") in ALS and 3 clusters in control using a Pearson correlation measure of expressed genes. Each gene cluster was assigned a putative cell type designation using a literature mining Python algorithm that cross-references genes in a given cluster with the names of spinal cord cell types. The absolute value of probe intensity for each gene within a given cluster was averaged together with other genes in that cluster to generate an "eipgen-gene" intensity for relative quantity of each predicted cell type. Cell types identified as increased in ALS include microglial, macrophage and astrocyte, while decreased populations included types 1 and 2 motoneurons. Cell types identified as increased in control patients include astrocytes, while decreased populations include type 2 motoneurons. Interestingly, both highly consistent and exact reciprocal patterns of gene cluster were observed. Especially, upregulated genes forming the cluster labeled as "microglial" or "macrophage" correlated best with the cluster formed by downregulated genes from the motoneurons cluster type 1 or 2 in ALS. Upregulated MHC class II genes correlated with these increased "glia", suggesting they were activated. Tissue staining confirmed significantly increased microglia/macrophage in connected motoneurons in sALS. Identifying interesting gene pathways is currently underway.

Conclusions and discussion: We utilized a novel bioinformatics analysis to identify microglia/macrophage activation in the region of lower motoneuron degeneration that are relatively remote in relation to the region of clinical onset in sALS. While the exact mechanisms of how activated microglia/macrophages promote neurodegenerative progression is not clear, the new findings support the hypothesis that neuro-glia physical interactions are important in the ALS pathologic process, and targeting microglia/macrophages could be a useful therapy for patients with sALS to slow disease progression.

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Session 9B Clinical Trials

C89 EFFICACY AND SAFETY OF EDARAVONE (MCI-186) FOR THE TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS (ALS): A 24-WEEK EXTENSION

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Keywords: edaravone, clinical trial, ALSFRS-R

Background: These are the results of a 24-week open-label (OL) extension period after the 24-week double-blind (DB) period (edaravone versus placebo) of a Phase III study. In the 24-week DB, edaravone was associated with less functional loss and quality of life deterioration than placebo (NCT01492686) (1)

Objectives: To assess the efficacy and safety of an additional 24 weeks of edaravone (E-E) versus DB placebo switched to OL edaravone (P-E) in ALS patients.

Methods: Patients entered DB with retained broad functionality (all ALSFRS-R individual item scores $\geq$2 points) and normal respiratory function (%FVC $\geq$80%) at baseline. DB completers could enter OL to receive active edaravone. OL was an additional 6 cycles of 60-mg intravenous edaravone once-daily for 10 of 14 days, followed by a 14-day drug-free period (28 days per cycle). The main efficacy endpoint was ALSFRS-R change across 12 cycles. Other endpoints included ALSAQ-40 scores and survival analyses for key ALS events (death and certain disease progression). Safety endpoints, including adverse events (AEs), were collected. The prespecified statistical plan called for descriptive statistics only.

Results: Of the 137 patients who entered 24-week DB, 123 patients entered 24-week OL; 65/123 (E-E) and 58/123 patients (P-E) were previously randomized to edaravone or placebo, respectively. In 24-week DB, edaravone showed less decrease in ALSFRS-R compared with placebo (between-group difference 2.49 ± 0.76 at 24 weeks, p=0.001). In 24-week OL, E-E group showed less observed change from study baseline in ALSFRS-R than P-E group (between-group difference 4.17 ± 1.40 at 48 weeks) (adjusted mean). Edaravone improved ALSAQ-40 with a difference between edaravone and placebo in 24-week DB (8.79 ± 4.03, p=0.031), and maintained the difference in 24-week OL (10.71 ± 4.51 at 48 weeks) (adjusted mean). Death or events of certain disease progression occurred in 10 and 19 patients in the E-E and P-E groups, respectively. The most commonly reported AEs ($\geq$5% of patients in both treatment groups) were nasopharyngitis, respiratory disorders, constipation, dysphagia, and contusion.

Discussion: Edaravone was favored at the 24-week endpoint of DB. During the next 24 weeks of OL, observed differences appeared to be maintained; E-E group appeared to show less functional loss than P-E group at 48 weeks. P-E group did not appear to "catch up" to E-E group who started edaravone 6 months earlier.

Conclusion: These observed findings suggest that early intervention with edaravone may confer a measureable benefit to individuals for whom treatment is promptly initiated versus individuals for whom treatment is delayed by 6 months (in ALS patients who meet inclusion criteria and continue edaravone through one year).

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C90 ADAPTIVE DESIGN SINGLE CENTER PHOSPHODIESTERASE TYPE 4 (PDE-4) INHIBITOR-(MN-166 (IBUDILAST)) PHASE 1B/2A CLINICAL TRIAL – INTERIM-BLINDED ANALYSIS – BEHAVIOR OF CREATININE AS A BIOMARKER IN SHORT CLINICAL TRIALS (NCT02238626)

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Keywords: randomized delayed drug initiation, clinical endpoint exploratory analysis, 6-month double-blind-open-label-extension

Objective: Interim blinded analysis of adaptive design for single center (MN-166 (Ibudilast)) phase 1b/2a clinical trial in ALS 1) no NIV (EC) and 2) NIV (ANC) patients.

Background: Ibudilast, effective in two ALS gene-based Drosophila models, has a known human safety profile that permits assessment of its effectiveness targeting multiple disease etiological pathways at different (early-distal axonopathy; late-microglial activation) ALS stages.

Methods: Clinical Endpoint Exploratory Analysis was performed by an independent statistical consult on 25 subjects with complete spirometry data who completed the DB-6-month clinical trial on MN-166 (Ibudilast)-plus-Riluzole (N=20) or Placebo-plus-Riluzole only (N=5). ALSFRS-R-total, -bulbar-subscore, -arm subscore, -leg subscore and -respiratory subscore were analyzed in addition to SVC. Clinical Endpoint Exploratory Analysis analyzed differences between MN-166 (Ibudilast) and placebo patients in clinimetric measurements. Directionality of treatment effect and sample size estimates for a 6-month clinical trial based on each Clinical Endpoint were analyzed at http://hedwig.mgh.harvard.edu/sample_size/size.html

Results: Clinical Endpoint Exploratory analysis pointed to effect sizes reducing required patients from 321 (ALSFRS-R-total-score) per group to 102 (ALSFRS-R-bulbar-subscore) or 84 (ALSFRS-R-arm-subscore) per group in 6-month clinical trials. El Escorial Criteria (EEC) clinically-definite ALS (cdALS) significantly (p=0.0001) increased during DB clinical trial (0 months = 2.9 (95% CI=2.73.1); 6 months = 3.3 (3.13.5)). Change in EEC in EC from clinically probable (cpALS) to cdALS during DB phase is significantly (p=0.028) proportionately higher (46.1%) in creatinine declining than not declining (11.1%) subgroups. EEC cdALS is significantly (p=0.005) proportionately higher in ANC (54.5%) than EC (12.9%) patients at clinical trial entry.

Conclusions: Ibudilast administration is feasible/tolerable/safe when administered over 3 months and 6 months to no-NIV ALS (EC) subjects. Ibudilast administration showed effect size estimates at 6 months in bulbar and arm clinical domains that will help define clinical endpoints for potential phase 3 clinical trials as well as pathways for adaptive change for this clinical trial. El Escorial Criteria disease burden increases significantly in ALS patients over 6 months and occurs significantly more in ALS patients showing decreased serum creatinine. Advanced NIV (ANC) ALS subjects have a higher proportion of El Escorial clinically definite disease and, as an enriched cohort, are safely participating in this clinical trial. The ANC patient group, enriched for cdALS, is potentially a vanguard for future clinical trials with a robust clinical trajectory that might be informative with respect to treatment effects on non-respiratory (bulbar, arm, leg) domains.

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C91 RASAGILINE FOR THE TREATMENT OF ALS: A RANDOMIZED CONTROLLED STUDY

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Keywords: rasagiline, mitochondrial biomarkers, ALSFRS-R

Background: Rasagiline, a monoamine-oxidase B inhibitor approved for the treatment of Parkinson's Disease may be neuroprotective in ALS by its action on stabilizing mitochondria. Rasagiline slowed disease progression in the SOD1 mouse model, and slowed the rate of progression on the ALS functional rating scale-revised (ALSFRS-R) in case series of patients with ALS. In our prior open label screening trial of rasagiline in 36 participants with ALS we did not show a difference in ALSFRS-R slope of decline compared to historical controls, but we did find rasagiline to be well tolerated and demonstrated possible mitochondrial biomarker engagement.

Objective: To determine whether rasagiline can slow disease progression and to validate a potential new mitochondrial biomarker in patients with ALS.

Methods: We conducted a 12 month randomized placebo-controlled clinical trial of 2 mg oral rasagiline compared to placebo at 10 medical centers in the United States between September 2012, and September 2016. Participants were randomized to rasagiline or placebo in a 2:1 ratio. Our primary outcome measure was the 12-month slope of decline of the ALSFRS-R. Secondary outcome measures included adverse event monitoring, mitochondrial biomarkers, vital capacity, a fall diary, and the ALS global quality of life question.

Results: One hundred and three patients were screened, and 80 were randomized. Participants were mostly male (78.8%) and the age range at baseline was 29–76 years of age. Currently, the clinical trial has completed enrollment,

and it is expected that the primary results will be available to present at the time of the meeting.

Conclusions: Rasagiline is a monoamine oxidase inhibitor which may be neuroprotective in patients with ALS. We conducted a randomized placebo controlled clinical trial to determine if rasagiline slows disease progression in ALS and to validate potential new mitochondrial biomarkers.

Clinicaltrial.gov identifier: NCT01786603

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C92 VITALITY-ALS, A PHASE 3 TRIAL OF THE FAST SKELETAL MUSCLE TROPONIN ACTIVATOR, TIRASEMTIV, FOR THE POTENTIAL TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS (ALS): STUDY DESIGN AND BASELINE CHARACTERISTICS

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Keywords: tirasemtiv, phase 3, VITALITY-ALS

Background: Tirasemtiv is a selective fast skeletal muscle troponin activator being evaluated for the potential treatment of patients with ALS. A large phase 2b trial of tirasemtiv (at a maximum tolerated dose up to 500 mg/day) versus placebo demonstrated statistically significant effects both on slow vital capacity and muscle strength after 3 months of treatment (BENEFIT-ALS) although not meeting its primary endpoint based on the ALSFRS-R. Tirasemtiv was most commonly associated with dizziness, fatigue, nausea, and slightly more weight loss than placebo. Although the open-label period successfully identified some participants who did not tolerate the drug, more participants dropped out of the study on tirasemtiv than on placebo. A phase 3 study, Ventilatory Investigation of Tirasemtiv and Assessment of Longitudinal Indices after Treatment for a Year (VITALITY-ALS; NCT02496767) was developed to confirm and extend the effects noted in BENEFIT-ALS. Additionally, several distinct strategies were implemented to improve the tolerability of tirasemtiv in participants entering the randomized phase of the trial.

Objectives: The primary objective of VITALITY-ALS is to assess the effect of tirasemtiv versus placebo on respiratory function in ALS.

Methods: VITALITY-ALS is a multi-national, double-blind, randomized, placebo-controlled, stratified, parallel group, study with tirasemtiv treatment up to 52 weeks in patients with ALS. Following 2 weeks of open-label tirasemtiv (125 mg BID), participants were randomized 3:2:2:2 to placebo or one of 3 target total daily dose levels of tirasemtiv (250, 375 and 500 mg). Eligible participants with a diagnosis $\leq$24 months and minimum SVC $\geq$70% predicted were enrolled from centers in North America and Europe. To reduce the rate of dropouts in the double blind phase of the trial, participants must successfully complete 2 weeks of open-label tirasemtiv prior to randomization compared with one week in BENEFIT-ALS. For participants randomized to higher doses than 125 mg BID (250 mg/day), dose escalation is also slower, with participants increasing their dose every two weeks rather than every week in BENEFIT-ALS. Participants are down-titrated if any signs of intolerance emerge. The primary outcome measure is change in SVC from baseline to 24 weeks. Key secondary outcomes include time to respiratory failure or death and time to a fall in SVC to $\leq$50%. Muscle strength, ALSFRS-R and survival will also be measured and a biomarker substudy conducted.

Results: Screening and enrollment began in September 2015; screening was completed in July 2016 and enrolment was completed in August 2016. Demographics and entry characteristics of the study population will be presented.

Discussion and conclusions: VITALITY-ALS tests the hypothesis that tirasemtiv will significantly reduce the decline of SVC over 24 weeks versus placebo, and that there will be a clinically meaningful impact on other measures of respiratory and skeletal muscle function.

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C93 CAN PYRIMETHAMINE LOWER CSF SOD1 LEVELS IN FAMILIAL ALS? RESULTS FROM A MULTICENTER PHASE II TRIAL

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Keywords: familial ALS, pyrimethamine, therapeutic trial

Background: Reduction of superoxide dismutase 1 (Cu/Zn-SOD1) levels prolongs survival in transgenic mouse models. Pyrimethamine was shown to produce dose-dependent Cu/Zn-SOD1 level reduction in cell culture systems. Previous clinical trials showed pyrimethamine lowers Cu/Zn-SOD1 levels in lymphocytes in patients with Cu/Zn-SOD1 mutations. This study investigated whether pyrimethamine could lower Cu/Zn-SOD1 levels in the cerebrospinal fluid (CSF) in patients with ALS associated with Cu/Zn-SOD1 mutations.

Methods: The study was multicenter (5 sites), open-label, 9 months duration, dose-ranging, to determine safety and efficacy of pyrimethamine to reduce Cu/Zn-SOD1 levels in the CSF of patients with a documented Cu/Zn-SOD1 mutation. All participants underwent 3 separate lumbar punctures, blood draw, clinical assessment of strength, motor function, quality of life, and adverse effects assessments. Both Cu/Zn-SOD1 and pyrimethamine levels were measured in the CSF. Blood was analyzed for pyrimethamine levels and Cu/Zn-SOD1 levels in red blood cells. Appel ALS, ALSFRS-R and single item McGill Quality of Life (SiS-MQoL) measures were also done at screening and at visits 6 and 9.

Results: Following approval from the Institutional review boards (IRB) at all the participating sites, 32 patients were enrolled in the study, 24 completed visits from screening through visit 6, 21 completed all study visits. 8 participants were unable to remain in the study long enough to complete visit 9. A generalized estimating equations (GEE) model was used to analyze the SOD1 levels in the CSF. This model showed a significant reduction in Cu/Zn-SOD1 levels at visit 6 ($p<0.001$) with a mean CSF Cu/Zn-SOD1 level reduction of 13.5% (95% CI $(-18.5, -8.4)$) and at visit 9 ($p<0.001$) with a mean reduction of -10.5% (95% CI $(-15.8, -5.2)$).

Conclusion: Pyrimethamine is a small molecule that is safe and well-tolerated in ALS. This molecule is capable of producing a significant reduction in Cu/Zn-SOD1 levels in FALS patients with Cu/Zn-SOD1 mutations.

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Session 10A Neuroinflammation

C94 THE CONTRIBUTION OF INFLAMMATION TO NEURODEGENERATION

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Keywords: neuroinflammation, microglial activation, neuronal function

It has been known for several years that neuroinflammatory changes, resulting primarily from microglial activation, was a characteristic of the aged brain and that similar, though perhaps more profound, changes also typified most neurodegenerative diseases. Whether these changes contributed to the associated developing loss of neuronal function was the subject of great debate. However recent findings, especially in the context of Alzheimer's disease (AD), have revealed that neuroinflammatory changes contribute to the pathogenesis of the disease. Consequently the need to understand the causes of the microglial activation and neuroinflammation is now identified as urgent because of the potential to identify novel therapeutic strategies for the treatment of early disease.

Microglia are maintained in a relatively quiescent state because of a predominantly anti-inflammatory milieu in the brain and because of the interactions between microglia and other cells; these strategies to maintain microglia quiescent are disrupted with age. Several homeostatic mechanisms shift with age and in a model of AD. These include the interaction between microglia and other cells by means of neuroimmune regulatory molecules and the increased infiltration of peripheral immune cells that cause activation of microglia, perhaps because of altered blood brain barrier function. The consequence of these changes is increased production of inflammatory cytokines from microglia, and also astrocytes, and these negatively impact on synaptic/neuronal function. I will present data indicating that mechanisms that control microglial function, that impact on cell interactions or infiltrating cells, have the ability to modulate neuronal function.

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C95 SUPPRESSING NEUROINFLAMMATION: A KEY TO THERAPY IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: neuroinflammation, Tregulatory lymphocytes, autologous transplant therapy

Background: Neuroinflammation is a prominent pathological feature of ALS with activated microglia and infiltrating T cells at sites of injury. In ALS neurons do not die alone; neuronal injury is non-cell-autonomous and depends on a well-orchestrated dialogue involving glia, T cells and motor neurons, actively mediating neuronal viability and neuronal injury. Data from ALS mouse models suggest that neurons inform microglia and T cells of the intraneuronal functional state, promoting neuroprotection with increased Tregs in early stages of disease, and neurotoxicity with decreased Tregs in later stages. Studies of ALS patients confirm a critical role for Tregs, with decreased Tregs and FoxP3 expression predictive of rapid progression and attenuated survival (1). A key question is whether Tregs are functional in ALS patients and whether they can be expanded and transplanted back into the same patient to slow disease progression.

Materials and methods: Lymphocytes were isolated from the blood of patients with definite ALS or from healthy controls. Following Treg (CD4+/CD25+) purification by Miltenyi columns, the ability of Treg to suppress Teffector cells (CD4+/CD25−) proliferation was assayed by both tritium and CFTE fluorescent techniques. Methylation assays of the Treg-specific demethylated region (TSDR) were performed on Treg DNA, followed by bi-sulphite treatment, PCR amplification, and sequencing. Tregs were expanded with anti-CD3/CD28 beads followed by incubation with IL-2 and rapamycin.

Results: ALS Tregs were less efficient in suppressing the proliferation of Teffs compared with control Tregs. The greater the burden of disease and the more rapid the disease progression in the ALS patient, the greater the Treg dysfunction. This dysfunction was a property of the ALS Tregs since suppression of ALS Teffs by control Tregs was not compromised. Epigenetically, the percent methylation of the TSDR was greater in ALS Tregs compared with control Tregs, with the more rapidly progressing ALS patients having greater methylation. ALS Tregs were readily expandable ex vivo. Following

expansion, the impaired suppressive functions of ALS Tregs were restored to levels of control Tregs.

Conclusions: Freshly isolated ALS Tregs were dysfunctional and less suppressive than control Tregs. Epigenetically, the reduced suppressive function of Tregs from ALS patients who progress rapidly may be due in part to increased methylation of the TSDR. Since the dysfunction was reversed following expansion, the in-vivo loss of suppression may result from serum or tissue factor modulation *in vivo* and not from irreversible alterations. Autologous adoptive transfer of ex vivo expanded Tregs offers a potentially novel cellular therapy for slowing disease progression in ALS, and a Pilot Safety Trial is now underway.

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C96 ACTIVATED IMMUNE RESPONSE IN THE PERIPHERAL NERVOUS SYSTEM IS INSTRUMENTAL TO DELAY THE DISEASE PROGRESSION IN ALS MOUSE MODELS

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Keywords: SOD1G93A mice, immune system, peripheral nervous system

The role of the immune system on the progression of amyotrophic lateral sclerosis (ALS) is still controversial being considered either pathogenic or beneficial depending on the context in which it is examined. Recently, we demonstrated that motor neurons (MNs) of C57SOD1G93A mice with slow disease progression activate molecules classically involved in the cross-talk with the immune system. This phenomenon occurs to a lesser extent in 129SvSOD1G93A mice that show a faster disease progression despite expressing the same amount of mutant SOD1 (1). Unexpectedly, neuropathological differences between the fast and slow progressing mice were not found in the loss of lumbar spinal MNs perikaria but rather in the axonal and neuromuscular compartments (2,3).

Objective: The present study investigated whether and how the immune response is involved in the preservation of motor axons in the mouse model of ALS with a less severe disease course.

Methods: The extent of axonal damage, Schwann cell proliferation and neuromuscular junctions (NMJs) denervation were compared between the two ALS mouse models at the disease onset using immunohistochemical and imaging techniques. Then we compared the expression levels of different immune molecules and the presence of blood derived immune cell infiltrates in the sciatic nerve of the two SOD1G93A mouse strains using immunohistochemical, immunoblot and qRT-PCR techniques.

Results: Muscle denervation, axonal dysregulation, myelin disruption together with a reduced Schwann cell proliferation is prominent in 129SvSOD1G93A compared to C57SOD1G93A mice. This correlates with the faster progression of the disease observed in the first strain. On the contrary, a striking increase of immune molecules, such as CCL2, MHCI and C3, was seen in sciatic nerves of slow progressor C57SOD1G93A mice and this was accompanied by heavy infiltration of CD8 + T lymphocytes and macrophages. These phenomena were barely or not detectable in the peripheral nervous system of fast progressing mice.

Discussion and conclusions: These data show for the first time that damaged MNs in SOD1-related ALS actively recruit immune cells in the peripheral nervous system in order to delay muscle denervation and prolong the lifespan. Thereby, the lack of this response has a negative impact on the disease course.

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C97 EARLY- AND LATE-ACTIVATED MICROGLIA SHOW DISTINCT LOCALIZATIONS AND EXERT DIFFERENT IMPACTS ON TDP-43 PATHOLOGY IN AMYOTROPHIC LATERAL SCLEROSIS SPINAL CORD

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Keywords: microglia, TDP-43, anterolateral funiculus

Background: In amyotrophic lateral sclerosis (ALS), marked microglial infiltrations are observed in anterolateral funiculus outside the corticospinal tract (ALFoc) of the spinal cord (1). Microglia express temporally distinct genes after activation (2) and, among them, osteopontin (Ost) is expressed in an early phase (4-24 hours), and galectin-3 (Gal3) in a later phase (3–7 days). Currently, relationships between temporal profiles of activated microglia and TDP-43 pathology in the motor neurons remains unknown in ALS spinal cord.

Objectives: This study aimed to detect to what extent early- and late-activated microglia contribute to this marked infiltration and ALS pathology.

Methods: Ten-percent buffered formalin-fixed, paraffin-embedded 5-μm-thick transverse spinal cord sections of sporadic ALS patients (n=7) and non-ALS patients (n=5) were examined immunohistochemically. The antibodies used were against Iba-1, CD68, Ost, Gal3, and TDP-43. Morphologies and distributions of immunoreactive (ir) cells were observed in the corticospinal tract (CST), anterolateral funiculus outside the CST (ALFoc), and anterior horn (AH). The numbers of IR cells in the 3 areas were quantified and subjected to correlation analyses.

Results: Distributions of Ost-ir cells were localized only in the AH and they had a rod or dot appearance. In contrast, Gal-3-ir cells populated the ALFoc and CST, and they had foamy profiles. The rates of Ost-ir/Gal3-ir to Iba-1-ir cells were 58.5/36.4%, and only the number of Gal3-ir cells in the ALFoc showed a significant correlation with that of motor neurons with a TDP-43 pathology (r=0.559, p=0.018), while the number of Ost-ir cells showed no correlations with the TDP-43 pathology. Iba-1-ir and CD68-ir cells were widely distributed in the ALFoc, CST, and AH.

Discussion and conclusions: This study clearly showed that in the ALS spinal cord, microglia activated in the later phase were present in the ALFoc and CST, and those activated in the early phase predominated in the AH, suggesting that microglia infiltration may occur in the white matter first and then spread to the AH. It was also implicated that Gal-3 inhibition might be a novel therapeutic target to protect motor neurons from ALS.

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C98 POST-PARALYSIS TREATMENT WITH MASITINIB SIGNIFICANTLY SLOWS DISEASE PROGRESSION IN TRANSGENIC SOD1G93A RATSPlatform Communications

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Background: Neuroinflammation and microgliosis are major neuropathological features of amyotrophic lateral sclerosis (ALS). Neuronal death and rapid paralysis progression are associated with the proliferation of activated glial cells with aberrant features (AbA cells). Previous studies have shown the importance of neuron-derived colony stimulating factor (M-CSF) via its receptor CSF-1R in microglial proliferation. Likewise, activated non-neuronal cells such as microglia and mast cells are known to produce a wide array of pro-inflammatory mediators with evidence of their upregulation found in ALS patients. Masitinib is a small molecule drug that selectively inhibits the tyrosine kinases of CSF-1R, c-Kit, LYN, FYN, and PDGFR in the nanomolar range. Masitinib provides a dual therapeutic approach by potently targeting microglia and mast cell activity, thereby potentially slowing disease progression and regulating neuroinflammation.

Objectives: We hypothesized that pharmacologic inhibition of CSF-1/CSF-1R signaling by masitinib treatment might prevent the appearance of aberrant glial cells, regulate neuroinflammation and slow paralysis progression.

Methods: Masitinib (30 mg/kg/day) (supplied by AB Science, France) was orally administered to symptomatic SOD1G93A rats immediately after paralysis onset (D1-cohort, n=14) or 7 days after paralysis onset (D7-cohort, n=9) and compared against matched vehicle-treated control groups (n=29). Glial cell pathology and motor neuron loss/atrophy were analyzed from spinal cord samples. Masitinib's impact on microglia neuroinflammatory components and migration, and emergence of AbA phenotype was assessed in primary cultured microglia from symptomatic SOD1G93A spinal cords.

Results: Survival probability for masitinib-treated rats when compared with controls was significantly different (D1-cohort p<0.0006; D7-cohort, p<0.0001). Mean post-paralysis survival time was 20 days for vehicle-controls versus 30 days (p<0.0016) and 27 days (p<0.0003) for the D1- and D7-cohorts, respectively. No gender difference was observed. Overall, masitinib significantly (p<0.01) reduced motor neuron loss by 34% compared with controls, significantly prevented reduction in soma diameter (30 versus 23 μm, respectively), and significantly reduced the number of AbAs in the lumbar spinal cord by 40%. *In vitro* masitinib significantly (p<0.01) inhibited M-CSF-induced microglia proliferation, significantly reduced the expression of several genes involved in inflammation processes by 80–50% (e.g. IL1β, IL6, Iba1, TNFα and Cox2), significantly inhibited by >50% microglia migration, and significantly inhibited by >50% the emergence of AbA phenotype microglia.

Discussion and conclusions: These data provide compelling evidence for masitinib's therapeutic potential in ALS, with treatment initiated 7 days after paralysis onset prolonging post-paralysis survival by 40% compared with controls. It is encouraging that recently published interim analysis data from a masitinib phase 3 randomized controlled trial reported success in all endpoints; thereby, substantiating these current data, which in turn provide a strong biological rationale for the observed clinical benefit in patients.

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C99 AN UNEXPECTED ROLE FOR MICROGLIA DURING RECOVERY FROM MOTOR NEURON DISEASE IN A NEW MOUSE MODEL OF TDP-43 PROTEINOPATHY

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Keywords: inflammation, TDP-43, reinnervation

Background: Though ALS is a disease in which motor neurons degenerate, it has long been appreciated that multiple other cell types could be involved in the disease progression. Our previous studies have characterized the disease course in rNLS8 mice following transgene induction of human TDP-43 in neuronal cytoplasm (hTDP43ΔNLS) by assessing axonal dieback, neuron loss, and the resultant motor dysfunction and death (1).

Objective: Having established the effects of mislocalized TDP-43 on neurons themselves, we next wanted to examine non-cell autonomous disease processes in rNLS8 mice.

Methods: We present a series of experiments in control and rNLS8 mice to examine the changes in the microglial response during disease (chronic hTDP43ΔNLS expression for 3 days, 2 weeks, or 6 weeks) and recovery (suppressing the transgene after chronic expression for 2 or 6 weeks) by immunofluorescence. We also manipulate the peripheral macrophages by the administration of liposomes and by irradiation-induced myeloablation and bone marrow transplantation. We characterize the functional role of microgliosis at the cell level by biochemistry and immunofluorescence and at the organismal level by behavior and muscle physiology.

Results: Surprising preliminary data showed no significant increase in microglial activation after hTDP43ΔNLS induction in the spinal cords of these mice, even in late disease stages. However, when we suppressed the transgene after 2 or 6 weeks of chronic expression, there was a dramatic increase in microglial activation concurrent with hTDP-43 clearance and axonal outgrowth from surviving motor neurons to reinnervate previously vacated neuromuscular junctions. Studies using bone marrow chimeric rNLS mice demonstrate that the increased microgliosis is the result of local proliferation rather than from infiltrating myeloid cells from the periphery. Early results also suggest that the activated microglia are responsible for the TDP-43 clearance, revealing a potentially important and currently underappreciated neuroprotective role. Functional studies investigating the effects of blocking this microglial proliferation on TDP-43 clearance and axonal outgrowth of motor neurons in early disease recovery are underway.

Conclusion: This work has important therapeutic implications as it challenges the hypothesis that microgliosis contributes to neurodegeneration, and rather suggests that the inflammatory response is required for recovery from motor neuron disease.

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Session 10B Disease Progression/Prognostic Modelling

C100 BASELINE PREDICTORS OF SURVIVAL IN A LARGE COHORT OF ALS PATIENTS: THE ALS COSMOS STUDY

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Keywords: survival, predictors, cohort studies

Background: Previous observational studies suggest that ALS survival is associated with factors such as increased age, smoking (in women), and site of onset; however, many of these studies did not recruit patients at the early stages of disease and did not prospectively follow them until death. Here, we report data from ALS COSMOS, a large prospective cohort of 355 ALS patients, recruited from major ALS centers in the US within 18 months of symptom onset and actively followed at 3, 6, 12, 18 and 24 months and thereafter followed passively.

Aims and methods: This paper evaluates predictors of survival in ALS COSMOS. Our ALS patient population is 60% male, with mean (standard deviation) age 61.1 (10.3) years. Predictors included sociodemographic and lifestyle variables, biomarkers (serum creatinine (SC), uric acid, 8-Oxo-2'-deoxyguanosine (8-oxo dg), total cholesterol and isoprostane), and a previously described measure of "good" nutrient intake. Survival time was calculated from time of symptom onset to death. Missing data were imputed using a fully conditional specification algorithm in SPSS (Version 23). We used Cox regression to estimate hazard ratios (HR) and their 95% confidence intervals (CI). All models included the ALS Functional Rating Scale - Revised and the Percent Forced Vital Capacity (FVC%) measured at baseline.

Results: No associations were found between any biomarker or between "good" nutrient intake and survival. Baseline ALSFRS-R and FVC% were strongly and independently associated with ALS survival in both univariate and multivariate analyses. In univariate analyses, we found that younger age, non-white race, Hispanic origin, reported smoking in the past 3 years, spinal (compared to bulbar) onset, and higher body mass index were associated with longer survival. Except for Hispanic origin and smoking, these associations persisted in a multivariate model. For each year increase in age, the estimated adjusted HR (95% CI) was 1.02 (1.01, 1,03); for whites, it was 1.51 (1.03, 2.23); for non-college degree education (compared to college or higher), it was 1.24 (0.97, 1.59); for bulbar (compared to spinal) onset, it was 1.33 (1.02, 1.74); and for each unit increase in BMI it was 0.98 (0,95, 1.01).

Conclusions: We find that younger age, non-white race, higher educational attainment and spinal onset were associated with longer survival among patients enrolled in ALS COSMOS. These findings can be used by clinicians to assess prognosis among newly diagnosed patients.

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C101 DEVELOPMENT AND EXTERNAL VALIDATION OF A PROGNOSTIC MODEL ESTIMATING SURVIVAL IN INDIVIDUAL ALS PATIENTS

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Keywords: prognosis, prediction, personalized medicine

Background: Survival of patients with amyotrophic lateral sclerosis (ALS) is highly variable and prediction of survival of individual ALS patients is currently largely unknown. This hampers individual risk-assessment, stratification of patients for trials, and timing of clinical interventions.

Methods: We performed an individual participant data meta-analysis of 11,471 ALS patients with a total follow-up time of 40,301 years, originating from 14 different research groups in 9 different countries. Based on predictors that were identified from previous studies and confirmed through variable selection techniques we developed a multivariable prognostic model that was externally validated multiple times using internal-external cross-validation. We calculated measures of discrimination and calibration.

Results: Eight variables entered the prediction model: site of onset (adjusted hazard ratio (HR) 1.24, $p<0.001$), age at onset (HR 1.03, $p<0.001$), definite ALS according to El Escorial criteria (HR 1.30, $p<0.001$), diagnostic delay (HR 0.97, $p<0.001$), forced vital capacity (HR 0.99, $p<0.001$), ALSFRS slope (HR 1.22, $p<0.001$), presence of frontotemporal dementia (HR 1.08, $p=0.385$), presence of a C9orf72 repeat expansion (HR 1.26, $p<0.001$). Our proposed model achieved good external predictive accuracy with a C-statistic ranging between 0.73 and 0.82 and a calibration slope between 0.70 and 1.31 (when implemented in future patients), indicating good agreement between predicted and observed survival probability.

Conclusions: This study proposes a model to reliably predict survival of individual ALS patients. This model can be used via a freely available and easy-to-use online tool. Results of this study bring individualized patient management and counseling, and future individualized trial design a step closer.

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C102 RETROSPECTIVE ANALYSIS OF DATA FROM A PHASE III TRIAL OF EDARAVONE IN AMYOTROPHIC LATERAL SCLEROSIS (ALS) USING TWO CLINICAL STAGING SYSTEMS

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Keywords: edaravone, staging, progression

Background: The King's ALS Clinical Staging System ("King's") and the ALS Milano-Torino Staging ("MITOS") System were first described in 2012 and 2013, respectively, as measures of disease progression in ALS (1,2). King's measures extent of anatomical involvement (upper limb, lower limb, bulbar, need for tracheotomy/gastrostomy), while MITOS captures loss of independence and function in four domains (movement, swallowing, communicating, breathing). In a Phase III clinical trial (Protocol MCI186-J19), edaravone was found to delay functional decline over 24 weeks as assessed by the Revised ALS Functional Rating Scale (ALSFRS-R) total score (3). Since King's and MITOS postdated this trial, we retrospectively examined the impact of edaravone in J19 using these staging systems.

Objective: To evaluate the potential utility of the King's ALS Clinical Staging System and the MITOS Staging System as measures of disease progression in ALS clinical trials.

Methods: Using patient-level data from J19, we retrospectively mapped ALSFRS-R item scores to King's and MITOS using published algorithms. In J19, 137 patients, aged 20–75 years, with definite or probable ALS, symptoms for $\leq$2 years, and forced vital capacity (FVC) $\geq$ 80%, were randomized to double-blind treatment with edaravone 60 mg/d (n=69) or placebo (n=68) for six 4-week cycles. At the end of double-blind treatment, all patients were offered 6 additional cycles of treatment with open-label edaravone; 65 edaravone patients and 58 placebo patients entered the extension study. We retrospectively examined progression in terms of any decline in King's, and $\geq$1-stage and $\geq$2-stage declines in MITOS, between baseline and weeks 24 and 48, respectively.

Results: Most patients (80%) in J19 were King's Stage 1 or 2 at study entry, and all were MITOS Stage 0. By 24 weeks, 42.0% (95% confidence interval (CI): 30.4%, 53.6%) of patients receiving edaravone had declined $\geq$1 King's stage vs 55.9% (95% CI: 44.1%, 67.6%) among those receiving placebo; corresponding figures for $\geq$1- and $\geq$2-stage declines in MITOS were 46.4% (95% CI: 34.8%, 58.0%) vs 47.1% (95% CI: 35.3%, 58.8%), and 2.9% (95% CI: 0.0%, 7.2%) vs 5.9% (95% CI: 1.5%, 11.8%), respectively. By 48 weeks, 72.5% (95% CI: 62.3%, 82.6%) of edaravone-to-edaravone patients, and

79.4% (95% CI: 69.1%, 88.2%) of placebo-to-edaravone patients, had experienced a ≥1-stage decline in King's; corresponding figures for ≥1-stage and ≥2-stage declines in MITOS were 66.7% (95% CI: 55.1%, 76.8%) vs 73.5% (95% CI: 63.2%, 83.8%), and 10.1% (95% CI: 4.3%, 17.4%) vs 23.5% (95% CI: 13.2%, 33.8%), respectively.

Conclusions: Our retrospective analysis suggests that both the King's ALS Clinical Staging System and the MITOS Staging System may be of value in evaluating the potential benefit of new therapeutic agents in ALS. These findings warrant further investigation.

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C103 ALSFRS-R PATTERNS OF DISEASE ONSET AND PROGRESSION THROUGH THE SPINE

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Keywords: ALSFRS-R progression, onset patterns, spinal regions

Background: ALS has traditionally been measured with ALSFRS-R total score, despite psychometric analyses suggesting a lack of unidimensionality.

Objective: To characterize the spread of ALS symptoms through neuronal regions from onset, and to identify recurring ALSFRS-R patterns to describe and help predict future progression.

Methods: We constructed progression trajectories using ALSFRS-R scores from PatientsLikeMe (PLM, N=3,995) and PRO-ACT (N=1,600). We reorganized the items into four weakly correlated "spinal groups" ("mouth", "upper limb", "lower limb", "breathing"), composed of highly correlated ALSFRS-R items. We normalized each patient's trajectory by rescaling the onset times and slopes of all the spinal groups according to the initial slope ("master slope") of the first spinal group to decline ("onset group"). Slope/onset patterns were modeled using mixed-effects and then used to predict ALSFRS-R scores 6 months in the future by estimating each patient's onset group and master slope from ALSFRS-R history and then projecting the associated progression pattern into the future.

Results: Similar slope/onset patterns were observed for both PLM and PRO-ACT patients. The slopes of spinal groups after the onset group were steeper than or similar to the master slope; and when one limb group was first to onset, the remaining limb group was next to deteriorate. When the progression patterns were used to predict ALSFRS-R score 6 months in the future, root mean square error was ∼6 points (out of 48) for prediction of PRO-ACT trajectories using patterns trained on PLM data only.

Discussion: Our approach estimated the extent of neuronal degeneration in different regions of the spine and to describe its spread and penetration over time. The progression patterns discovered here suggest that ALS accelerates as it spreads, regardless of the initial velocity of the disease; and that how it spreads through the spine depends upon where it started. Classification of patient trajectories by onset group and degree of fit to the spinal model provide opportunities for trial inclusion criteria based on how "typical" a patient's disease appears to be, suggesting fewer anomalies, higher likelihood of trial completion/survival, and ultimately increased statistical power. Our model could be further refined by considering other covariates (age, sex, etc.) as well as other descriptors of disease history (fit error of historical trajectory). Alternative onset types could be investigated. Our method could take full advantage of future versions of the ALSFRS-R which could provide more detail regarding disease location (e.g. left vs. right side, hand vs. arm weakness).

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C104 AUTONOMIC DYSFUNCTION IN ALS. SYMPATHETIC OVERACTIVITY PREDICTS VELOCITY OF DISEASE PROGRESSION

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Keywords: autonomic dysregulation, disease progression, prognostic factor

Background: Several autonomic nervous system (ANS) disturbances have been observed in ALS. No clear correlation between ANS involvement and ALS clinical characteristics has been found so far.

Aim: The aim of this study was to correlate the results of power spectral analysis of RR interval and systolic arterial pressure (SAP) variability with patients' clinical features.

Methods: Fifty-two ALS patients (age 61.79 ± 11.47, 28 men) and 16 healthy subjects were included. We recorded (i) disease duration (DD); (ii) functional status using the ALSFRS-R score and its bulbar subscore; (iii) rate of disease progression (RDP) expressed in ALSFRS-R loss

per month. All subjects were studied at REST and during TILT. We extracted HP and SAP beat-to-beat series. HP was defined as the temporal distance between two consequent R-wave peaks. Inside each HP the maximum of arterial pressure signal was taken as SAP value. Time domain indexes such as mean and variance of HP (μHP and 2HP, respectively) and of SAP (μSAP and 2SAP, respectively) were calculated. Parametric power spectral analysis was performed on both HP and SAP series. According to its central frequency, each component was classified as low frequency (LF) or high frequency (HF). Absolute power of HP and SAP series in LF band (LFa,HP and LFa,SAP) and in HF band (HFa,HP and HFa,SAP) were assessed. Power in HF band of HP series is considered an index of the vagal modulation directed to the heart, while LFa,SAP is an index of the sympathetic modulation directed to the vessels. Linear correlation analysis was performed to verify the association between ALS clinical features and the indexes extracted from HP and SAP series.

Results: Both at REST and during TILT ALS patients were more tachycardic than controls. μHP decreased during TILT in both groups. 2HP and LFa,HP were significantly lower in ALS than in controls in both conditions. 2HP and HPa,HP in ALS patients decreased significantly during TILT. 2SAP and LFa,SAP increased during TILT only in controls, while both indexes were significantly smaller during TILT in ALS patients. The results of the correlation analysis of the calculated indexes and the ALS clinical features showed that only the associations of RDP on LFa,SAP at REST and on HFnu,HP during TILT were significant (p=0.02 and p=0.03 respectively).

Discussion and conclusions: We found that (i) all ALS patients were characterized by an impairment of the autonomic regulation both at level of cardiac and vascular controls; (ii) the impairment is more evident in the vascular than in the cardiac control; (iii) spectral indexes of the cardiovascular regulation are significantly correlated with the rate of the disease progression, and not with its severity; (iv) fast progressors showed higher cardiovascular sympathetic modulation compared to slow progressors.

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C105 MAKING SENSE OF THE ALSFRS-R USING JOINT LONGITUDINAL AND SURVIVAL MODELS OF FUNCTIONAL DIMENSION SUB-SCORES

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Keywords: progression, prognosis, sub-groups

Introduction: ALSFRS-R is the most widely used functional rating system in ALS patients. Despite this, heterogeneity in ALSFRS-R progression, and longitudinally informative censoring due to patient drop-out/mortality, cause difficulties in analysis. In addition, latent functional dimension sub-scores have been identified in the ALSFRS-R score.

Methods: All cases with ALSFRS-R scores on the Irish ALS register were included. Functional ALSFRS-R sub-scores were defined for bulbar, motor and respiratory domains. Joint longitudinal and survival models were used to visualise fitted total and sub-score ALSFRS-R progression. In addition the prognostic value of convenience and computed ALSFRS-R slope and sub-score slopes were compared.

Results: 407 cases were identified with valid ALSFRS-R scores – 233 (57%) were male, 125 (31%) had bulbar onset disease. Graphs of joint model fit demonstrated correction for non-informative censoring in total ALSFRS-R score. There was evidence that spinal and bulbar sub-scores reached minima within 3 years in spinal and bulbar onset patients respectively. Site of onset was predicted using ALSFRS-R bulbar and motor sub-scores, with 90% sensitivity and specificity.

Discussion: Our analysis builds on previous knowledge of ALSFRS-R sub-scores. Joint longitudinal and survival models were able to correct for drop-out/mortality of total ALSFRS-R. We further demonstrate that ALSFRS-R bulbar and motor sub-scores are important prognosticators and capture much of the variability in progression due to the disease site of onset. Our analysis indicates that decline in ALSFRS-R motor sub-scores may begin in a prodromal phase of the disease in spinal patients.

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C106 IN SILICO BLOCK RANDOMIZATION OF ALS PATIENTS USING A MACHINE LEARNING ALGORITHM

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Keywords: randomization, predictive analytics, survival prediction

Background: Small, randomized trials can have meaningful differences in treatment arms. Stratified randomization reduces this risk. In ALS, riluzole use (RU) and bulbar onset (BO) are used for stratification. We hypothesized that stratified randomization could be improved with predicted survival as a single stratifier.

Objectives: Defining a randomization failure as a statistically significant difference between treatment arms on one baseline characteristic, we aimed to compare failure rates in randomization stratified by RU and BO ("traditional stratification") to randomization stratified by a survival prediction algorithm in simulated trials using the PRO-ACT database.

Methods: We first selected the 4,482 records in PRO-ACT with ALSFRS and FVC. Our survival prediction algorithm used Gradient Boosting Machine (GBM) log

likelihood of survival (GBM package in R) calculated from baseline patient characteristics. The 4,482 records were randomly divided into ten (n= ~448) pools. To derive our survival prediction algorithm for each pool and avoid overfitting, we trained the model on records from the nine other pools. Thus, we created 10 algorithms - one to be applied to each pool. Each of the 10 pools was further divided into 2, 4, 7 or 10 virtual trials, with 224, 112, 64 or 44 records, respectively. For the smallest virtual trials (n=44), this yielded 100 iterations (10 pools with 10 virtual trials). Survival predictions were made for each patient. The resulting log likelihoods were used to assign a percentile rank to each patient record within a virtual trial. Randomization strata were created based on these percentiles. In order to identify the best split for strata, four schema were explored: (i) < 20th percentile, 20–90th, and >90th; (ii) < 25th percentile, 25–75th, and >75th; (iii) < 33rd, 33–66th, and >66th; and (iv) < 50th and >50th percentiles. Patient records were randomized to one of two trial arms using 100 randomization schedules. For comparison, the same virtual trials were randomized using traditional stratification (RU/BO). Rates of randomization failure were compared in the traditional stratification and each of the four stratification schema using the algorithm.

Results: Survival algorithm stratification had fewer failures than traditional stratification. Of the four algorithm stratification schemas, the <25th, 25–75th, and >75th appeared superior. The traditional stratification successfully randomized RU and BO, but the algorithm stratification failed less frequently overall. Furthermore, because this was an *in silico* experiment, patient outcomes were known and could be compared. The algorithm randomization demonstrated fewer failures when comparing outcome measures, including ALSFRS-R and FVC slopes and survival.

Conclusion: Using a survival prediction algorithm to stratify randomization appears more likely to create balanced randomization in small ALS trials.

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C107 RATE OF CHANGE AND LINEARITY OF ALSFRS-R AND ITS SUBSCALES IN THE PRO-ACT DATABASE

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Keywords: ALSFRS-R, PRO-ACT, DeltaFS

Background: The rate of change of ALSFRS-R is an important outcome in clinical trials. However, its linearity has been questioned. There is little information on relative rates of decline of subscores of ALSFRS-R.

Objective: The Pooled Resource Open-Access ALS Clinical Trials (PRO-ACT) database was examined to address the following: (1) to determine if trajectories of ALSFRS-R and its subscores are linear or curvilinear, and (2) to study differences in change of subscores, depending upon recorded site of onset, as markers of topographic spread.

Methods: Mixed models were fitted to obtain individual rates of decline, or "post-slope". For the total ALSFRS-R score, the correlation between rate of decline from onset of symptoms ("pre-slope") and post-slope was examined. Additional previously reported determinants of post-slope were confirmed. Models with linear and quadratic time terms were fitted to examine non-linearity for the total score and subscores. For each site of onset (bulbar, arm and leg), time to reach a 25% drop in each subscore (or a subscore of 9) was estimated from linear models using bootstrap.

Results: As of January 2016, PRO-ACT includes data from 10,723 individual patients pooled from 23 clinical trials. Of these, repeated scores and time from date of onset are available for ALSFRS-R in 3367 cases, bulbar, fine motor, and gross motor subscores in 6116 cases, and respiratory subscore in 3,065 cases. Mean ALSFRS-R post-slope was −0.99 points/month (SD 0.71). Although the pre-slope had a highly significant effect on post-slope, the correlation was modest (0.4). Post-slope was steeper with bulbar-onset disease (−0.24 point difference). Respiratory subscore decline was less steep than that of other subscores. There was evidence of significant non-linearity in trajectories of ALSFRS-R and each of its subscores, the effect being most striking for respiratory and motor subscores. ALSFRS-R trajectories were overall concave, although for the steepest tertile of pre-slope, they were slightly convex. Subscore thresholds of 9 points were crossed at time point estimates in months, by site of onset in the following order: Bulbar-onset (bulbar 9.3, gross motor 18.4, fine motor 19, and respiratory 28.6); Arm-onset (fine motor 6.1, gross motor 17.5, bulbar 29, and respiratory 36.9); Leg-onset (gross motor 5.6, fine motor 19.3, respiratory 32.6, and bulbar 33.5).

Conclusion: Significant non-linearity in the decline of ALSFRS-R subscores over time is confirmed and has important implications for trial design. The pre-slope is an imperfect predictor of the post-slope. Evolving changes in subscores of ALSFRS-R recapitulate clinically observed topographical spread of ALS.

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Session 11 Joint Closing Session

C108 AIRLIE HOUSE ALS CLINICAL TRIALS GUIDELINES WORKSHOP 17-19 MARCH 2016

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Keywords: guidance, guidelines, regulatory science

Background: The genesis for the Airlie House ALS Clinical Trials Guidelines Workshop 2016 lay in marked variations in the approaches of the regulatory agencies: Food and Drug Administration (FDA-USA) (www.fda.gov) (1), European Medicines Agency (EMA-EU) (www.ema.europa.eu) (2,3), and Pharmaceuticals and Medical Devices Agency (PMDA-Japan) (www.pmda.go.jp/English) (4,5) in developing guideline recommendations for ALS clinical trials.

Methods: Each current ALS Clinical Trials guideline (6) was reviewed. Each current guideline was accepted as is, modified, or rejected by the key topic area group by voting using the Delphi Method. For each research question, the summarized background/evidence/rationale was presented at the Reporting Session of the Workshop. Proposed guideline/recommendation(s) to each research question were presented. After the Workshop, the final proposed guidelines will be posted on a secure website and the final voting process will be completed using the modified Delphi Method (a maximum of 2 weeks). The final guidelines will be provided (via website or e-mail) to the ALS community for public commentary. (This will be done between the second and third round of voting of the modified Delphi process. This will give the groups a chance to make changes in response to comments and re-vote on those changes).

Conclusions: In an effort to meet the gaps in developing treatments for ALS patients, an international meeting was convened at Airlie House, the seat of the original Consensus ALS Clinical Trials Guidelines (6). This project was designed to forge modern guidelines for clinical translational scientists, pharmaceutical, bioengineering and device companies, as well as regulatory agencies in the USA, Europe and Japan with an evidence-informed Expert Consensus Process reviewed independently by a Modified Delphi Method subsequent to the conference that was aired online.

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C109 ENTERING THE ERA OF PRECISION MEDICINE: REALIZING THE VALUE OF MND DATA AT SCALE

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Keywords: precision medicine, biomarker, genomics

Precision medicine offers delivery of optimally targeted, precisely timed interventions tailored to an individual's molecular drivers of disease. Precision medicine as a concept is only just beginning to be considered for treating amyotrophic lateral sclerosis (ALS). ALS is a complex, multifactorial disease, compounded by biological complexity, genetic risk factors and heterogeneity in each individual's susceptibility and phenotype. For effective treatment interventions at population or individual level to be developed, unequivocal understanding of its clinical and biological complexities need to be established. There are significant challenges. Precision intervention requires that the there be a process of comprehensive risk assessment, clear definition of causative mutation, establishment of genetic risk, understanding of the interaction of genes with environment, and definition of latent pathophysiological processes. These then require testing in order to develop and provide tailored treatment. But current approaches to understand environmental and genetic risk are confounded by a lack of power, as single-center studies usually lack sufficient statistical power to assess environmental risk and genetic risk is limited to understanding of cases of familial ALS.

Large scale genomic assay of patients is offering the potential of new insight into this problem from the perspective of genetic variation at scale, but with such scale comes the challenge of managing hugely increased dataset sizes, together with the responsibility of sharing data and intellectual resources so that the most rapid progress possible can be achieved. The promise of precision medicine applied at the individual level needs to be met with the development of *de facto* de-personalization of scientific endeavour to highly co-ordinated collaborative consortia. Successfully pioneered by the Psychiatric Genomics Consortium which consists of united investigators around the world conducting meta- and mega-analyses of genome-wide genomic data, the mega-consortium approach can yield startling numbers of genes that provide new insights into the mechanisms, coupled functional studies and rapid development of prototype interventions. The combination of individual groups or closed consortia into a truly coherent, open sharing community, so that the multiple facets of precision medicine approaches can be combined, remains the single most challenging hurdle to the realization of precision medicine for ALS.

We present a pilot project that embraces the consortium approach to support broad systems level analyses together with functional studies and genomic background to yield a promising prognostic biomarker for ALS.

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C110 A PRECISION MEDICINE APPROACH TO ALS: WHAT WILL IT TAKE?

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Keywords: precision medicine, heterogeneity, genetics

ALS is a complex disease, with a large heterogeneity both in term of clinical features and pathogenetic mechanisms. The conceptual definition of ALS as a disease of the motor system (upper and lower motor neurons) does not hold anymore, after the observation of the involvement of other neuronal systems, in particular the frontotemporal areas, leading to various degrees of cognitive impairment, and the identification of the functional impairment of basal ganglia, at least in a subgroup of patients. ALS heterogeneity spreads over different domains, i.e. phenotype, genetics, molecular mechanisms, pathogenesis, with the possible single common denominator of TDP43 pathology. This complexity is a serious obstacle toward the identification of an effective cure for ALS.

How is it possible to have a rational approach to this complexity? Some clues derive from the demonstration that the Armitage-Doll model, originally proposed for carcinogenesis, also holds for ALS. According to this model, ALS can be explained by a 6-step process, i.e. a sequential series of 6 events that convert a healthy "motor neuron-glia complex" to a harmful "tissue environment" causing motor neurons death (1). In patients carrying a mutation of a gene related to ALS, the number of steps is reduced, proportionally to the penetrance of the specific gene, indicating that a genetic mutation shortens the "path" to motor neuron degeneration; however, also in presence of a strong genetic background, other factors, either genetic or environmental or both, are necessary to complete the degenerative process.

The challenge of the next years will be therefore to identify the factors that are involved in this sequential degenerative process. We have already a few clues, i.e. some "minor" genes that modify the phenotype of ALS, such as UNC13A, ATXN2 and CAMTA1; biochemical mechanisms, such as patients' lipid profile; environmental factors, such as cigarette smoking; but most of the mechanisms are still to be identified.

However, precision medicine is already a reality in ALS. The recent implementation of a phase 1B clinical trial on antisense oligonucleotides (ASO) in SOD1 patients represents the first step for a molecular targeted treatment at least in a subgroup of patients (2). A similar therapeutic approach is expected to start the next year for patients carrying the C9orf72 hexanucleotide expansion, which is

by far the commonest known gene in ALS in Caucasian populations.

Further progresses in the direction of precision medicine for ALS are predictable with the ongoing efforts aimed at genome sequencing of DNA from large cohorts of patients (Project MinE), and at the set-up of large biobanks of Induced Pluripotent Stem Cells obtained from clinically and genetically well-annotated ALS patients (Genomic Translation for ALS Clinical Care).

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