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Session 1 Joint Opening Session

C1 ADDRESSING THE BURDEN OF NEURODEGENERATIVE DISEASE: A GLOBAL CHALLENGE

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Keywords: non-communicable diseases, neurodegenerative diseases, neurodisability

Neurologists across the world deal with various disorders of the brain and the peripheral nervous system with vast numbers of individuals affected. The human and financial cost of neurodegenerative disorders is immense. The scientific and clinical care cost will be beyond the abilities of all of us by the year 2050.

It is most encouraging that at this moment in time neuroscientists are working hard to understand the basic pathology of neurodegenerative diseases. This is absolutely fine but the natural progression would be clinical application and therapeutic trials would be the next step, if we were to find a disease modifying therapy or even a cure. In the recent ministerial meeting on dementia at the WHO (April 2015) it was made clear that such trials are difficult to conduct and require time and commitment. It is also clear that researchers and clinicians cannot pursue one or two disorders without getting into the pathophysiology of several interconnected neurodegenerative processes. The pathological processes involving alpha synuclein, tau and amyloid go beyond specific clinical diagnoses and have to be looked at in such a manner. Open research is what is being called for now and joining efforts not only in finding positive outcomes but also to avoid going down the same route previously looked at by others. We need to supplement and not duplicate research. This will move us in a more targeted and faster manner.

Neurological non-communicable diseases (NCDs) including dementia will bankrupt health budgets across the world. This crucial message should be hammered home at all levels for those working in neurology and those responsible for health budgets, so that the necessary resources can be allocated now to avoid this inevitable situation. The WFN is engaged in the combat of non-communicable neurological diseases and is ready to provide the global platform for this task.

No action is not an option.

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C2 IS ALS A MULTISTEP PROCESS?

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Keywords: epidemiology

Amyotrophic lateral sclerosis (ALS) has several intriguing features which remain unexplained. It is an adult-onset disorder even in those born with a gene mutation that increases ALS risk; disease onset begins suddenly and progresses rapidly; several ALS genes show pleiotropy; and ALS seems to start in one neural region and spread. These features are consistent with the hypothesis that ALS involves a multistep process (1).

Multistep models have previously been used in cancer, but have not been used in ALS until recently. These models yield predictions which are consistent not only with the above features, and with other aspects of the epidemiology of ALS including: (i) there is a linear relationship between log(incidence) and log(age); (ii) the slope of 5 indicates that ALS is a six-step process; (iii) the slope (log(incidence) vs log(age)) is lower in those with a family history of ALS, indicating that they have inherited at least one step; (iv) the slope also differs by site of onset (with spinal having a lower slope than bulbar), age at onset, and duration of survival, indicating that these variables may identify distinct patient subgroups with differing numbers of steps involved in their disease; in particular, there is a lower slope for older-onset ALS with long duration survival, indicating that this subgroup of patients may be clinically different.

The model is also consistent with some aetiological theories of ALS (eg long-term aggregation of protein) but not others (eg the commonly used dose-dependent two-step process of genetic risk and environmental triggers). The model also allows the possibility that some steps may be the same across different neurological disorders, and the sequence or nature of the exposures needed to induce the relevant steps would mean that different outcomes arise, thus explaining genetic pleiotropy. Thus, some other neurological conditions (eg frontotemporal dementia) may involve similar multistep processes, whereas others (eg multiple sclerosis) do not.

If ALS involves a multistep process, this has profound implications for our understanding of disease mechanisms, the interplay of risk factors, and the further research that is needed to identify the steps. It implies that environmental exposures may be important (and their effects preventable) even in those with inherited mutations that increase ALS risk. It also raises hope that the identification of the steps could lead to preventive and therapeutic measures, including better patient stratification.

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Session 2A RNA Biogenesis and Processing

C3 THE ROLE OF LOW COMPLEXITY DOMAINS IN PROTEINS INVOLVED IN ALS

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Keywords: amyloids, hydrogels, RNA biogenesis

The vast majority of DNA and RNA regulatory proteins consist of two parts. One such part enables direct recognition of DNA or RNA, is well folded, and is represented by canonical domains including zinc fingers, homeoboxes, leucine zippers, RNA recognition motifs, KH domains and pumilio domains. The other part is typified by poorly folded, low complexity sequences whose mechanistic basis of function has long been enigmatic. When incubated at high concentration, certain of these low complexity domains can polymerize into amyloid-like fibers. My presentation will outline studies indicating that low complexity sequence polymers may represent the organizational basis for the formation of nuclear and cytoplasmic puncta including nuclear speckles, P granules, stress granules and neuronal granules. It is our speculation that from the birth of a transcript in the nucleus to its ultimate translation in the cytoplasm, the entire pathway of information flow is guided by movement of the message through a solid state pathway of polymeric fibers. This "informational cytoskeleton" is regulated in a dynamic manner by post-translational modification including phosphorylation, and can be impinged in disease states via mutational events that either enhance fiber stability or clog the dynamic behaviour of puncta.

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C4 GAIN OF TOXICITY FROM HEXANUCLEOTIDE EXPANSION IN C9ORF72 IN ALS AND FRONTOTEMPORAL DEMENTIA IS ALLEVIATED BY ANTISENSE OLIGONUCLEOTIDES TARGET REPEAT-CONTAINING RNAS

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Background: An abnormal expanded hexanucleotide repeat (GGGGCC) in intron 1 of chromosome 9 open reading frame 72 (C9orf72) is the most common genetic abnormality in familial ALS and FTD (1). This sense repeat expansion (GGGGCC) and its corresponding antisense repeat expansion (CCCCGG) result in bidirectional transcription and repeat-associated non-ATG (RAN) translation, causing the production of six dipeptide repeat proteins (DPRs) (2). Dipeptide repeat protein aggregates are seen in different parts of CNS including disease and non-disease related areas (3).

Objectives: To achieve a more comprehensive evaluation of DPRs in the brain and spinal cord of a C9 mouse model, we determined if DPRs can be found in this mouse model and if their burden is greater in disease-related regions. We also determine if size and burden of DPRs aggregations varies with *C9orf72* expression level or if it is age-dependent.

Methods: We generated several lines of transgenic mice expressing a human *C9orf72* gene with 450 repeats. Using immunohistochemistry and immunofluorescence, we are evaluating the presence of 5 different DPRs: GA, GP, GR, PA, and PR in various regions of brain and spinal cord of a C9 mouse model, including mice with different expression levels of *C9orf72* and mice with different ages.

Results: We found that DPRs can be detected in different regions of brain and spinal cord of a C9 mouse model and their burden is more in areas unrelated to disease. We also found that the size and burden of these aggregations increased with the age of the mice and is more in homozygous mice compared to heterozygous mouse with same age.

Discussion and conclusions: Increased burden and size of aggregations with age and level of expression in a C9 mouse model can be a sign that these aggregations are age and expression level dependent. The fact that the aggregations are more prevalent in areas not related to disease can be a sign that insoluble DPRs are not contributing to disease pathology.

Acknowledgements: We would like to thank Cleveland's and Lagier-Tourenne lab that let us to have access to tissues of this C9 mouse model.

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C5 NEUROPATHOLOGY IN C9ORF72-ALS IS CONSISTENT WITH PURA SEQUESTRATION AND LOSS OF FUNCTION IN MOTOR NEURONS DESPITE COMPENSATORY OVEREXPRESSION

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Keywords: C9ORF72, immunohistochemistry, PURA

Introduction: GGGGCC repeat expansion of *C9ORF72* represents the most common genetic variant of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Currently the mechanism of pathogenesis is unknown but it is suggested that gain-of-function toxicity may be caused by RNA foci transcribed from the repeat sequence. We and others have determined protein binding partners of these RNA foci, including PURA, which may be sequestered leading to a relative loss of their normal function. Loss of PURA function has been previously linked to neurodegeneration and mislocalisation of axonal proteins.

Method: Pathological material from *C9orf72*-ALS patients was obtained from the Sheffield Brain Tissue Bank. Sense and antisense RNA foci were visualized by RNA fluorescence in-situ hybridization (FISH). Colocalisation of RNA foci with PURA, PURA levels and distribution of axonal proteins was examined by immunohistochemistry (IHC). Furthermore, alteration in the levels of PURA in spinal cord of *C9orf72*-ALS patients was validated by western blotting.

Results: PURA was shown to bind RNA foci derived from the GGGGCC-repeat expansion. Blinded study of PURA in motor neurons revealed higher expression in *C9orf72*-ALS cases which was confirmed by western blot. Despite PURA up-regulation, IHC markers of PURA function, including distribution of phosphorylated neurofilament, suggest a functional inhibition.

Discussion: We hypothesise that sequestration of PURA by RNA foci inhibits PURA function despite attempted compensation through up-regulation. PURA overexpression might therefore rescue toxicity.

Acknowledgements: We acknowledge grants from EU Framework 7 (Euro-motor), and the JPND/MRC SOPHIA, STRENGTH and ALS-CarE projects. JCK and JRH hold MND Association/MRC Lady Edith Wolfson Fellowship awards. Biosample collection was supported by the MND Association and the Wellcome Trust (PJS). We are grateful to those who donated biosamples.

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C6 RBM45 HOMO-OLIGOMERIZATION MEDIATES ASSOCIATION WITH ALS-LINKED PROTEINS AND STRESS GRANULES

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Keywords: RNA-binding protein, RBM45, homo-oligomerization

Background: The aggregation of RNA-binding proteins is a pathological hallmark of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). Structure-function studies of TDP-43 and FUS have provided considerable insight into the mechanisms by which these and other RNA-binding proteins exert their normal functions, associate with cytoplasmic stress granules, and aggregate into inclusion bodies in disorders such as FTLD and ALS. Our lab has identified a new RNA-binding protein, RBM45, with pathologic alterations in ALS and FTLD. RBM45 containing cytoplasmic inclusions were observed in both ALS and FTLD patients (1). RBM45 also co-localized with TDP-43 and ubiquitin inclusions in affected neurons. Recently, our lab reported a role of RBM45 in the antioxidant response in ALS (2). However, the structure-function of RBM45 and how it contributes to the progression of neurodegenerative diseases remains unclear.

Objective: To further define the role of RBM45 in ALS and FTLD, we identified RBM45 amino acid domains that regulate its function, subcellular localization, and interactions with itself and ALS-linked proteins.

Methods: We used *in vitro* culture cells (HEK293, Neuro2A and SK-N-SH) as models. Biochemical, molecular and cellular biology techniques were used for this study.

Results: We determined that RBM45 forms homo-oligomers and physically associates with the ALS-linked proteins TDP-43 and FUS in the nucleus. Nuclear localization of RBM45 is mediated by a bipartite nuclear-localization sequence (NLS) located at the C-terminus. RBM45 mutants that lack a functional NLS accumulate in the cytoplasm and form TDP-43 positive stress granules. Moreover, we identify a novel structural element, termed the homo-oligomer assembly (HOA) domain, that is highly conserved across species and promotes homo-oligomerization of RBM45. RBM45 mutants that fail to form homo-oligomers exhibit significantly reduced association with ALS-linked proteins and inclusion into cytoplasmic stress granules.

Discussion: We define the structural determinants of RBM45 required for its nuclear import and interactions with cytoplasmic stress granules and other proteins linked to ALS, including TDP-43 and FUS. Our results show that RMB45 functions as a homo-oligomer and that its oligomerization contributes to ALS/FTLD RNA-binding protein aggregation. Future mechanistic studies of RBM45 are warranted to further define the roles of RBM45 in neurodegeneration, which will broaden therapeutic options for ALS and FTLD.

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Session 2B Clinical Management

C7 MULTIDISCIPLINARY CARE IN ALS: MEASURING THE IMMEASURABLE?

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Keywords: multidisciplinary care, outcome, measurement

A quality healthcare system is co-produced by patients, families and healthcare professionals working interdependently to co-create and co-deliver care. Optimal clinical decision-making combines experience and judgement, scientific evidence and patient preferences and values, while also recognizing the psycho-social context of both the patient and the health care professional.

In general, it is the objective of health care professionals working in ALS to guide those affected towards optimizing the timing and utility of interventions, maximizing quality of life, while also attempting to support in the later stages the prospect of a "peaceful death" using a symptom based approach. By applying these principles, successful multidisciplinary clinics achieve an improvement in survival and quality of life for patients. Measurement of the functionality of the multidisciplinary clinic and the model of multilevel decision-making is challenging, and the precise mechanisms by which these improvements are achieved have not been fully characterized. Moreover, there have been few detailed analyses of the perspective of the patient and the carer, their expectations of care, or their attitudes towards specialist rather that generalist clinical care of ALS, and their compliance with specialist recommendations. Many patients also continue to experience delays in accessing multidisciplinary clinics, as general neurologists may continue to manage ALS patients as individual practitioners rather than as integrated members of a specialist multidisciplinary service. Similarly, some patients elect not to attend specialist clinics despite being aware of the evidence base in favour of survival and quality of life. From a healthcare professional perspective, there is an imperative to explore the important but poorly addressed areas of burnout, moral distress, and ethical/existential discordance between providers and services users.

New methods are required to understand the range and pattern of decision-making practices associated with a successful multidisciplinary ALS clinic. These will help to generate models of care that reflect integrative decision making and enhanced service utilization in the context of recent advances in clinical staging and cognitive and behavioural status of the patient, carer burden, and professional wellbeing.

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C8 THE EXPERIMENTAL TREATMENT OF BULBAR SYMPTOMS IN ALS WITH NUEDEXTA

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Keywords: ALS treatment trial, bulbar function, assessment of speech and swallowing

The Nuedexta treatment trial was initiated to follow-up anecdotal reports that bulbar symptoms were ameliorated in patients treated for pseudobulbar affect. Since it was observed that a treatment effect was relatively rapid, a crossover design was chosen to minimize the number of subjects. After random assignment, 60 subjects were treated for one month with Nuedexta or a placebo and subsequently switched, after a wash out period, to a second one month course of treatment with either drug or placebo, as appropriate. The study was conducted at seven clinical research sites.

Outcome measures were recorded at a baseline visit and at the beginning and end of each one month trial period. The Center for Neurologic Study-Bulbar Function Scale (CNS-BFS)[1], a self-report scale, was the primary outcome measure. Secondary outcome measures included timed speech and swallowing[2], the ALSFRS-R[3], and the use of visual analogue scales.

The mean CNS-BFS score for the treatment group was 53.66 versus 58.67 in the placebo group, a statistically significant improvement ($p = 0.0001$). All three domains of bulbar function that were evaluated-speech, swallowing, and salivation responded positively to treatment. Approximately half the patients exhibited pseudobulbar affect (PBA). Treatment effect was independent of the patients' PBA status. Both speech rate and swallowing time responded to treatment, although neither was statistically significant.

In conclusion, Nuedexta palliates bulbar symptoms in patients with ALS. More extensive studies will be required to determine the duration of the treatment effect, and the possibility that Nuedexta exerts disease-modifying effects.

Acknowledgements: Supported by the ALS Association TREAT ALS Award for Pilot Clinical Trials.

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C9 TREATMENT OF MEDICALLY REFRACTORY SIALORRHEA WITH ELECTRON BEAM RADIOTHERAPY (EBRT) TO THE PAROTID

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Keywords: sialorrhea, parotid, radiotherapy

Background: Sialorrhea is a troublesome symptom that negatively affects quality of life in ALS. Since the estimated saliva production in adults is 0.75 to 1.5 L/d, drooling can be a contributing factor to dehydration as well. In a small case series, we reported our initial positive experience with unilateral parotid EBRT to treat sialorrhea (1).

Methods: Radiotherapy was administered unilaterally to the parotid gland using a linear accelerator to produce an external electron beam. The dose was 1500 cGy delivered in 3 equal fractions. Electron energy was targeted according to the parotid depth mapped by 3D CT scanning.

Unstimulated unilateral saliva production was measured in 10 patients by placing individually weighed dental rolls between the lower teeth and the buccal mucosa. Measuring saliva production simultaneously on both sides was better tolerated. After 5 minutes, the dental rolls were removed and weighed. Saliva production was determined by difference. Patients continued their previous treatment but reduced medication as tolerated following EBRT.

Results: We treated 32 ALS patients, 19 females (59.4%) and 13 males (40.6%), age 66.5 ± 8.9 years (mean $\pm$ SD). The interval between disease onset and EBRT was 27.5 ± 7.6 months and the ALSFRSr score was 23.8 ± 7.6. The %FVC was 26.1 ± 21.0 although 50% were unable to perform FVC measurements due to poor lip seal. All patients had severe bulbar weakness and 75.0% (24/32) had bulbar-onset disease. Two had prior treatment with parotid botulinum toxin injections and 4 (12.4%) were not taking any anticholinergics due to side effects or lack of efficacy. Two received bilateral EBRT and 1 (3.1%) received a second EBRT treatment on the contralateral side. Baseline saliva production was 2.54 ± 1.2 g/5min. Within the first month, saliva production was reduced to 0.45 ± 0.34 g/5min which was maintained over 22 months post-EBRT (0.16 ± 0.07 g/5min). EBRT was well tolerated and long-term complications such as xerostomia were not encountered.

Conclusions: EBRT is safe, effective, and long-lasting symptomatic treatment of sialorrhea in ALS. Clinically, improvement was noted as early as 2 weeks following EBRT. Anticholinergics were able to be reduced or discontinued. At our institution, if anticholinergics are ineffective or not tolerated ALS patients are treated with EBRT as the intervention of choice.

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C10 A NATIONAL STUDY OF MUSCLE CRAMPS IN AMYOTROPHIC LATERAL SCLEROSIS

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Background: Muscle cramps are a problematic symptom in ALS. The ALSCARE database reports the occurrence of cramps in up to 87% of ALS patients. Despite its common occurrence as a symptom, research on cramps has been limited, but pain as a symptom of ALS has emerged as a theme.

Objectives: To describe the frequency, severity and temporal nature of cramps to ALS disease progression. To examine the relationship of cramps to pain intensity, interference with daily life and pain behavior. To report on treatments used for cramps in ALS.

Methods: The study used an anonymous public online survey and recruited participants registered with the Agency for Toxic Substances and Disease registry (ATSDR) National ALS Registry. Demographics, ALS Functional Rating Scale–Revised (ALSFRSR), cramp severity, frequency and treatments, and the National Institute of Health Patient Reported Outcomes Measurement Information System (PROMIS) pain questions for intensity, interference and behavior were collected. Descriptive statistics, analysis of variance (ANOVA) and Pearson product moment correlations were used to evaluate the data.

Results: 282 ALS participants completed the study (93% Caucasian, 64% male, mean age 58 ± 9.7years, mean disease duration 52 ± 48 months). Cramps were a symptom of ALS for 92% of participants, and was the first symptom experienced by 20%. Participants reported an average of 5.3 cramps per day (range 0-75) and a mean VAS cramp severity rating of 5.2/10. Compared to disease onset; 48% reported that their cramps increased, 23% indicated no change and 29% reported a decrease in frequency over time. There was no correlation between disease duration and cramp counts. Of participants, 47% did not seek treatment for cramps, while 17% used over-the-counter remedies and 36% used prescription medications. Those who used prescription medications reported

the highest cramp counts, severity, and PROMIS pain outcome scores compared to the other two groups. Cramp counts were significantly correlated with pain intensity ($r = 0.32$, $p < 0.001$), pain interference ($r = 0.31$, $p < 0.001$), and pain behavior ($r = 0.30$, $p < 0.001$). Stronger correlations were observed when examining cramp severity with the three pain outcomes ($r = 0.67$, $r = 0.54$, and $r = 0.55$, all, $p < 0.001$).

Discussion: This study represents the largest observational report of muscle cramps in ALS. Cramps are common and their severity is moderate. In this series, no temporal relation of cramps to disease progression or duration was observed. A high proportion of participants did not treat their cramps, while those who received treatment continued to have frequent and severe cramps. A portion of respondents had muscle cramps as their only source of pain and in this group the pain outcome instruments measured cramp pain alone. This study underscores the clinical importance of muscle cramps in ALS. Research is in progress to further explore cramp symptom management from patient and physician perspectives in order to develop better guidelines for care.

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Session 3A RNA Localization and Dysregulation

C11 ALTERED RNA METABOLISM IN ALS AND RELATED DISEASES

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Keywords: stress granules, phase separation

Pathological redistribution of RNA-binding proteins (RBPs) from the nucleus to cytoplasmic punctae is a common feature of a specific subset of age-related degenerative diseases, including ALS, FTD, IBM and MSP. These RBPs share in common intrinsically disordered low-complexity domains (LCDs) that are a frequent site of disease-causing mutations. We previously showed that such mutations accelerate fibrillization of RBPs and drive hyperassembly of stress granules. However, the mechanism whereby LCDs in RBPS contribute to the assembly of stress granules and related RNA-protein assemblies is unknown; nor is it understood how the hyperassembly of stress granules contributes to the deposition of insoluble, fibrillar aggregates of RBPs in disease. Here we show that hnRNPA1 has the intrinsic ability to self-assemble through weak, multivalent interactions that lead to the formation of phase separated, liquid-like droplets. RNA is recruited by hnRNPA1 into these droplets and facilitates phase separation that happens at more physiological protein concentrations. These droplets recapitulate the dynamic features of stress granules. By domain mapping, we demonstrated that the LCD of hnRNPA1 is responsible for phase separation. Interestingly, we found that the disease-causing mutant form of hnRNPA1 forms fibrils in the droplets, suggesting that phase separation increases the risk of fibrillization. We propose that phase separation of RNA- binding proteins through their low-complexity domains represent the mechanism leading to the recruitment of proteins to stress granules. The abundance of moderately aggregation-prone low-complexity domains in stress granule components, coupled with prolonged high local concentrations of such proteins together, may eventually lead to the formation of amyloid-like structures that could result in persistent stress granules. The higher propensity of proteins containing disease-causing mutations to fibrillize likely leads to the pathogenicity and cytoplasmic inclusions observed in patients.

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C12 NUCLEAR PORE ABNORMALITIES IN C9ORF72 ALS IPS NEURONS AND TISSUE ALTER NUCLEOCYTOPLASMIC PROTEIN TRAFFICKING

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Keywords: C9ORF72, nucleocytoplasmic trafficking, iPS

Background: A 'GGGGCC' repeat expansion in the *C9orf72* gene is the most common known genetic cause of familial and sporadic amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) (1-3). The *C9orf72* GGGGCCexp RNA can sequester nuclear factors, including RNA binding proteins, and we hypothesize that these aberrant interactions are a major contributor of *C9orf72* neurotoxicity. Work from our laboratory has indicated that the GGGGCC RNA interacts with RanGAP1, a regulator of Ran-mediated nucleocytoplasmic protein trafficking that resides on the cytoplasmic filaments of the nuclear pore complex (NPC) (4).

Objectives: To determine whether expression of a GGGGCCexp RNA species disrupts NPC function and nucleocytoplasmic trafficking in *C9orf72* ALS/FTD neurons.

Methods: To employ genetic screening of a G4C2 expressing *Drosophila* model and fixed and dynamic imaging of iPS neurons from *C9orf72* ALS/FTD patients to identify NPC abnormalities and nuclear trafficking deficits.

Results: Consistent with a RanGAP1 loss-of-function model, the G4C2 expressing *Drosophila* model shows reduced nuclear localization of NLS-containing reporters including TDP-43. iPS-neurons from *C9orf72* ALS patients exhibit perturbed nuclear/cytoplasmic Ran protein gradient and reduced nucleocytoplasmic import rates a reporter containing a classical NLS. Importantly, these nuclear transport deficits and protein mislocalization can be rescued by treating the C9orf72-patient derived iPS-neurons with antisense oligonucleotides that target GGGGCC repeat-containing RNAs. Antisense oligonucleotides (ASOs) or small molecules that bind G-quartet RNA structures also rescue neurotoxicity in the

Drosophila model. Supportive of a dysfunctional nuclear pore complex that underlies *C9orf72* ALS pathogenesis, we also observe pathologic staining of nuclear pore proteins and RanGAP1 in the motor cortex of *C9orf72* ALS patients. Moreover, we show that the toxic GGGGCCexp RNA disrupts nuclear import of classical NLS-containing reporter proteins and TDP-43.

Discussion and conclusion: We show evidence of nucleocytoplasmic trafficking deficits in a G4C2 expressing *Drosophila* model and in iPS neurons from *C9orf72* ALS/FTD patients. These deficits can be rescued by targeting the G4C2 RNA. Dynamic imaging of *C9orf72* ALS/FTD iPS neurons also reveal impaired nucleocytoplasmic import rates and enhanced cytoplasmic TDP-43 protein. Genetic modifiers of the nuclear import pathway or targeting the G4C2 RNA also rescue neurodegeneration in the G4C2 expressing *Drosophila*. NPC pathology can also be observed in *C9orf72* ALS/FTD patient motor cortex. Taken together, these studies strongly support a gain-of-function mechanism underlying the C9orf72-mediated neurodegeneration that affects NPC function and nucleocytoplasmic trafficking.

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C13 CHARACTERIZATION OF C9ORF72 EXPRESSION IN PATHOLOGICAL COHORT UNCOVERS NEW CLINICAL ASSOCIATIONS WITH SPECIFIC C9ORF72 TRANSCRIPTS

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Keywords: C9ORF72, expression, genetics

Background: To date, repeat expansions in chromosome 9 open reading frame 72 (*C9ORF72*) represent the most frequent genetic cause of frontotemporal dementia (FTD) and motor neuron disease (MND); however, the effect of this expansion on *C9orf72* expression levels has yet to be assessed thoroughly.

Objective: To characterize *C9orf72* expression in a unique pathological cohort of expansion carriers in order to help elucidate underlying mechanisms.

Methods: Our study cohort consisted of *C9orf72* expansion carriers (n=56), FTD or MND patients without expansions (n=31), and control subjects without neurological diseases (n=20). In the cerebellum and frontal cortex, we measured *C9orf72* transcripts (ie variant 1 (V1), variant 2 (V2), and variant 3 (V3)) as well as intron 1 containing transcripts with quantitative real-time PCR (Taqman) and digital molecular barcoding (NanoString Technologies) techniques. Data was normalized to the geometric mean of neuronal markers.

Results: In the cerebellum, we observed a 42% reduction in total *C9orf72* transcripts in expansion carriers as compared to control subjects (p-value=1.6e-07), while a 28% reduction was detected in the frontal cortex (p-value=2.3e-06). When evaluating individual transcripts, the most profound differences were encountered for V2, with a 57% decrease in the cerebellum (p-value=1.3e-06) and 42% in the frontal cortex (p-value=1.1e-05). We also noted a decrease in V1: 53% in the cerebellum (p-value=9.0e-06) and 31% in the frontal cortex (p-value=0.001). Importantly, there was no significant difference for V3 in the cerebellum or frontal cortex. When comparing expansion carriers to FTD or MND patients without expansions, we also discovered a reduction in *C9orf72* transcripts (ie total, V1, and V2). Interestingly, within our group of *C9orf72* expansion carriers we observed significant associations with survival after onset. The strongest association was noted for V1 in the cerebellum (hazard ratio=0.31; p-value=0.003) and frontal cortex (hazard ratio=0.23; p-value=0.0001), suggesting that higher levels prolong survival (using 25th percentile as cut-off point). Excitingly, our data also showed that intron 1 containing transcripts were more frequently detected in the frontal cortex of expansion carriers than in (disease) controls (p-value $\leq$0.003), which could reflect intron retention and/or truncated transcripts. Moreover, there was a significant difference in intron 1 containing transcripts between disease subgroups (ie FTD, FTD/MND, and MND; p-value=0.002) that appeared to be driven by the presence of FTD.

Discussion and conclusions: We are the first to present a detailed assessment of *C9orf72* expression in a pathological cohort of *C9orf72* expansion carriers. We report a significant reduction in *C9orf72* transcripts, especially in V1 and V2 transcripts, accompanied by an increase in intron 1 containing transcripts. Importantly, we also reveal a significant association with survival after onset, indicating that higher *C9orf72* levels may have beneficial effects, which could have major implications for the development of new therapeutic approaches targeting *C9orf72* (eg antisense oligonucleotides (ASOs)).

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C14 IN-DEPTH ANALYSIS OF SENSE AND ANTISENSE RNA FOCI IN A LARGE PATHOLOGICAL COHORT OF C9ORF72 EXPANSION CARRIERS

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Keywords: C9ORF72, RNA foci, RNA FISH

Background: An expanded repeat in chromosome 9 open reading frame 72 (*C9ORF72*) is causative of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Although the formation of RNA foci is thought to contribute to the disease, detailed investigation of RNA foci has not been performed in a large pathological cohort of expansion carriers.

Objectives: In order to unravel the mechanisms underlying *C9ORF72*-related diseases, we visualized RNA foci in brain tissue of *C9ORF72* expansion carriers using RNA fluorescent in situ hybridization (FISH).

Methods: Our study cohort consisted of 50 *C9ORF72* expansion carriers for whom cerebellum and frontal cortex were available. We performed RNA FISH on formalin-fixed paraffin-embedded (FFPE) tissue using probes targeting sense foci (Exiqon), antisense foci (Exiqon), and individual *C9ORF72* mRNA transcripts (Stellaris). Five μm thick sections were obtained, and hybridized overnight to Exiqon probes at 80°C or to our Stellaris probe at 37°C. Following hybridization, slides were imaged with an Axio Imager Z1 microscope (Zeiss) and a series of Z-stack images was acquired. In the cerebellum, over 1000 cells were scored for each patient, whereas in the frontal cortex 100-200 cells were scored.

Results: We detected nuclear RNA foci containing sense and antisense transcripts in both the cerebellum (~20% of neurons) and frontal cortex (~40% of neurons). In the cerebellum, nuclear RNA foci were predominantly observed in Purkinje cells and granular cells. Interestingly, antisense RNA foci were much more abundant in Purkinje cells (>5 RNA foci per cell) as compared to granular cells (1-2 RNA foci per cell); on the other hand, sense RNA foci were more frequently encountered in granular cells. In the frontal cortex, nuclear RNA foci were also distributed along neurons and glial cells of the gray matter; the frequency of RNA foci did not differ between neuronal and glial cells, and there was no obvious difference between sense and antisense foci. Excitingly, we were also able to visualize *C9ORF72* transcripts using novel RNA FISH techniques, which revealed less *C9ORF72* mRNA transcripts in *C9ORF72* expansion carriers as compared to ALS or FTD patients without these expansions, aligning with the decrease in *C9ORF72* that has been reported using quantitative real-time PCR; we observed the most striking reduction in Purkinje cells.

Discussion and conclusion: We performed an in-depth characterization of a unique pathological cohort of *C9ORF72* expansion carriers. Our results show that RNA foci are more common in the frontal cortex than in the cerebellum. Although no clear differences were noted between cell types in the frontal cortex, antisense RNA foci were more frequently encountered in Purkinje cells, whereas sense foci seemed more prominent in granular cells. These cell- and region-specific differences, most certainly, help to clarify the pathological hallmarks of *C9ORF72*-related diseases and to unravel its underpinnings.

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Session 3B Holistic Care

C15 DISCUSSING PROGNOSIS WITH ALS/MND PATIENTS: BALANCING HOPE AND REALISM

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Keywords: communication, prognosis, decision making

Background: Understanding prognosis, in terms of time and disease trajectory, is crucial for informed decision making yet many patients with serious illness struggle to integrate prognostic information and sometimes do not seem to understand what they have been told (1).

Objectives: To describe how patients cope with prognostic information and review best communication practices for talking with patients about prognosis.

Methods: We reviewed expert recommendations for discussing prognosis with seriously ill patients.

Results: The majority of work has been published in oncology but can be generalized to patients with serious illness, including those with ALS/MND. When discussing prognosis, experts carefully titrate how much to say and emphasize the importance of supporting hope.

Discussion and conclusion: Communication about prognosis is an iterative process that should begin early in the disease trajectory and be tailored to patient preferences. Patients with ALS/MND have a unique disease trajectory characterized by functional loss and by executive and behavioral impairment; discussion of prognosis should prepare patients for these outcomes. Future work should focus on determining optimum timing of discussions in order to prepare patients and families for decision making.

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C16 EXPERIENCES OF PEOPLE WITH MOTOR NEURONE DISEASE (MND) AND THEIR FAMILY CAREGIVERS RECEIVING THE NEWS OF THE DIAGNOSIS: A NATIONAL SURVEY

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Keywords: breaking bad news, satisfaction with support, empathy from neurologist

Background: Breaking the news of the diagnosis of MND is challenging for both neurologists and patients. The manner in which the patient learns of the diagnosis has implications for the way that patients and families move on from the devastating news of their diagnosis to the actions required for support throughout the illness trajectory.

Objectives: of this study were to identify the experiences of people with MND and their family caregivers in receiving the diagnosis and to determine which aspects of the process of breaking the news were associated with greater satisfaction.

Method: consisted of an anonymous postal survey facilitated by all MND Associations in Australia, in 2014. Questions centred on the SPIKES protocol for communicating bad news.

Results: provided a comprehensive insight from the patients' (n=248, response rate 29%) and family caregivers' perspective (n=194): 36% were dissatisfied with the delivery of the diagnosis and gave lower ratings on the ability/skills of their neurologists. It was evident that the longer the patients spent with their neurologists during breaking such bad news, the more they were satisfied with the delivery process and the higher they rated the neurologists' ability/skills. The largest significant differences in performance between the two groups of neurologists (with low and high ratings of skills) were in four domains: Responding empathically to the feelings of patient/family; sharing the information and suggesting realistic goals; exploring what patient/family are expecting or hoping for; making a plan and follow through. It is worth noting that family carers reported similar experiences to the patients.

Conclusions: This national study has detailed where practices in breaking the news of an MND diagnosis can be improved from the patients' perspective. With nearly 40% of patients dissatisfied with their experience of the diagnosis delivery, there is room for improvement in the practice of neurologists in Australia.

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C17 THE IMPACT OF FAMILY ON DECISION-MAKING IN ALS CARE: THE PATIENT PERSPECTIVE

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Keywords: family care-giving, care preferences, decision-making in care

Background: Caring for a person with ALS can have an adverse effect on family caregivers' psychological well-being. Studies have shown that families of people with ALS encounter high levels of care burden and report distress when they feel unsupported by healthcare services. The majority of studies that have investigated the impact of caring on family in ALS care have captured experiences from the carer's perspective. Few studies have reported on the role of family in ALS care from the patient perspective.

Objectives: To identify how ALS patients perceive the role of the family caregiver in ALS care and how they relate to their family in shaping their experiences of care.

Method: We employed an inductive qualitative approach (1) to explore the impact of family on ALS care, from the patient perspective. We undertook in-depth qualitative interviews with a diverse group of ALS patients (n=34) from across the Republic of Ireland whom we had recruited from the Irish ALS population-based register. Interviews comprised open-ended questions which encouraged participants to communicate at length about their experiences of care and how they perceived family impacted on their care.

Results: Participants accepted, declined and/or delayed services at the request of their family even when they differed from family in their preferences for care. Participants indicated that reliance on family to provide care had the potential to create conflict between them and their family. Nonetheless, participants felt obliged to engage with symptomatic and life-sustaining interventions, to alleviate family anxiety and provide emotional support to their family. Alongside participants' awareness of the care and support that they received from family, participants were committed to supporting family members even when their own physical strength and capacities were waning. The majority of participants perceived informal flows of family support and care as bi-directional.

Discussion and conclusions: Family can exert a central influence on health and social care preferences of ALS patients. Attention to ALS patients' supportive roles within their family is important in light of the strong focus on 'caregiver' care burden in current ALS research and practice. Family care-giving in ALS can be reciprocal: people with ALS receive care from family but also seek to reassure their family and make decisions about care in the interest of their family. The findings highlight the need for service providers to support ALS patients when patients themselves render emotional support to their family. ALS patients also encounter 'care burden' because their expressed care preferences and consequently their care outcomes are often shaped by the obligations they feel towards family.

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C18 DETERMINANTS OF CAREGIVER STRAIN IN CARERS OF PATIENTS WITH ALS; A LONGITUDINAL STUDY

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Keywords: caregiver strain, longitudinal cohort, multidisciplinary care

Background: Informal caregivers of patients with ALS are vulnerable as caregiving exerts substantial strain that increases during disease progression. To optimize caregiver support, insight is needed into potentially modifiable factors associated with caregiver strain.

Objectives: To examine the longitudinal associations between caregiver strain and patient disease characteristics and patient and caregiver psychosocial characteristics.

Methods: At 4-monthly intervals during a one-year follow-up, data on caregiver strain and patient and caregiver factors potentially associated with caregiver strain were collected in a cohort of 126 patients with ALS (without cognitive dysfunction) and their primary informal caregiver, who participated in a RCT on the effectiveness of case management (1). Caregiver strain was assessed with the Caregiver Strain Index (CSI). Patient and caregiver factors included socio-demographic characteristics, distress (HADS), coping style (Utrecht Coping List domains Passive Reaction and Active Approach) and perceived quality of care (Numeric Rating Scale), as well as the patient's functional status (ALSFRS-R) and emotional functioning (ALSAQ-40 domain Emotional Functioning). We investigated the longitudinal relationship of these time-independent and time-dependent factors with caregiver strain by using random coefficient analysis. Factors were added 1 by 1 to the basic model to study their individual relationship with caregiver strain. Finally, all variables significantly ($p < 0.10$) related to caregiver strain were simultaneously entered into the multivariate regression model using the backward elimination method, leading to a resulting model with significant determinants for caregiver strain ($p < 0.05$).

Results: Random coefficient analyses showed that caregiver strain increased significantly during the study period ($\beta = 0.315$ points/months, $p < 0.001$). Univariate analyses resulted in five caregiver variables (age at inclusion; HADS anxiety and depression; quality of care for the caregiver; UCL Passive Reaction) and seven patients variables (age at inclusion; ALSAQ-40 domain Emotional Functioning; ALSFRS-R sum score; quality of care for the patient; HADS depression; UCL Active Approach and Passive Reaction) that were significantly associated with caregiver strain. The final model showed that the patient's functional status ($\beta = -0.131$ points/months, $p < 0.001$) and emotional functioning ($\beta = 0.022$ points/months, $p = 0.03$), and the caregiver's factors passive coping style ($\beta = 0.152$ points/months, $p=0.03$), symptoms of anxiety ($\beta = 0.186$ points/months, $p < 0.001$) and perceived quality of care for the caregiver ($\beta = -0.452$ points/months, $p < 0.001$) were independently associated with caregiver strain.

Discussion and conclusions: Our study has identified that apart from the patient's physical disability and emotional well-being, a passive (avoidant) coping style of the caregiver, increased symptoms of anxiety and feeling less supported by the ALS-team impact on the strain of ALS caregivers. Professionals involved with the care of patients with ALS should be aware of these factors and increase their attention for the caregiver. This knowledge may guide the development of supportive interventions that focus on caregiver's coping style and avoiding distress.

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Session 4A Disease Models

C19 GENERATING NEW MOUSE MODELS OF ALS

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Keywords: mouse, CRISPR/CAS9, mutations

The genomic and biologic conservation between mice and humans, along with our ability to manipulate the mouse genome, places the mouse as a premier model for deciphering disease mechanisms and testing potential new therapies. Despite these advantages, mouse models for ALS have been relatively scarce and those that have been used most successfully present some interesting challenges that must be carefully addressed. Unlike many Mendelian diseases for which mouse models have been most successfully utilized, ALS is a complex disease. Clinical heterogeneity in patients is significant with variations in the rate of progression, age of onset, and bulbar vs. limb onset, as well as the degree of upper and lower motor neuron involvement. While the final common end result is motor neuron loss, a multitude of pathways are implicated, including RNA processing, excitotoxicity, protein misfolding and aggregation, oxidative stress, axonal transport and mitochondrial function. Added to this complexity is that the majority of ALS cases are sporadic in nature (sALS); thus the underlying genetic causes have been largely unknown, giving researchers limited insight into disease modelling, biological pathways and mechanisms.

However, with new advances in sequencing and genome editing technologies, a new path has emerged. This path involves more precise and rigorous approaches that enable the identification of clear genetic and/or biological subgroups within this sporadic disorder. Already, the *g*enome sequencing of large numbers of ALS patients has led to invaluable insight into the genetic contributions of ALS. One can now imagine a scenario where a very specific treatment for each type of ALS is approached in these defined subgroups. And, the identification of these disease contributing variants is timely, as the CRISPR/Cas9 genome editing system offers tremendous opportunity to reproduce pathogenic mutations found in humans in animal models. The simplicity of this technique also allows us to induce the same mutation in different genetic backgrounds, allowing us to address disease heterogeneity in unprecedented ways with advantages that go beyond the speed of generating the mice. But with powerful technology comes great responsibility. What models does the ALS community have, what models are being made, what is needed, and most importantly how do we harness these resources in a way that allows us to maximize the potential of these new innovations?

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C20 NEW MOUSE MODELS OF ALS SHOW NEURON SURVIVAL AND MOTOR RECOVERY AFTER CLEARANCE OF TDP-43 PATHOLOGY

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Keywords: TDP-43, mouse models, neuropathology

Background: Phosphorylated cytoplasmic TDP-43 aggregates in the brain and spinal cord are the molecular hallmarks of ALS. However, the role of this TDP-43 pathology in the pathogenesis of ALS remains unclear.

Objectives: 1) To generate transgenic mice with cytoplasmic human TDP-43 (hTDP-43) expression in the brain and spinal cord, in order to produce a model which recapitulates disease pathology and a progressive ALS-like phenotype; 2) To investigate whether or not mice could functionally recover when TDP-43 expression was suppressed late in the disease course?

Methods: New transgenic mice expressing the tetracycline transactivator (tTA) protein under control of the *NEFH* promoter were produced and crossed to mice with hTDP-43 harbouring a defective nuclear localization signal (ΔNLS) under the control of the tet-operon (1). Expression of hTDP-43ΔNLS was induced by doxycycline withdrawal and TDP-43 expression was suppressed by returning mice to doxycycline. TDP-43 levels, phosphorylation and solubility were analyzed by immunoblotting. Phospho-TDP-43, gliosis, neuron loss, brain and muscle atrophy, and muscle denervation were analyzed by immunohistochemistry/immunofluorescence. Disease progression was monitored by body mass, hindlimb clasping, tremor, rotarod and wirehang tests and survival. Three *NEFH*-tTA lines were characterized with similar results. $n \geq 3$ were used for biochemistry and imaging and $n \geq 10$ were used for behaviour studies respectively. $P < 0.05$ was considered statistically significant, as determined by two-tailed t-tests or ANOVA with Bonferonni's post-test.

Results: Bigenic mice expressed hTDP-43ΔNLS in the brain and spinal cord from 1 week after doxycycline withdrawal, with accumulation of cytoplasmic insoluble phospho-TDP-43. The mice developed limb tremor and hindlimb clasping at 2-3 weeks, with progressive decline in motor task performance. Brain and muscle atrophy began at 4 weeks, and spinal cord motor neuron loss occurred at 6 weeks, with weight loss leading to disease endstage at a median of 10.3 weeks. When hTDP-43ΔNLS expression was suppressed at 6 weeks, a time point when ~25% of lumbar motor neurons were lost and over 50% of tibilais anterior neuromuscular junctions (NMJs) were denervated, TDP-43 pathology was cleared, neuron loss was

halted, NMJs were reinnervated, and dramatic motor improvement occurred with prevention of death.

Discussion and conclusions: These new *NEFH*-hTDP-43ΔNLS mice delineate the timeline of pathology development, muscle denervation and neuron loss in ALS and demonstrate that removal of TDP-43 pathology is beneficial even in late stage disease. These findings indicate that future therapeutics targeting TDP-43 dysfunction are likely to be beneficial even in ALS patients with TDP-43 pathology where significant neuron loss has occurred and disease has progressed.

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C21 A GAIN OF TOXICITY BY C9ORF72 GGGGCC EXPANSION IN AMYOTROPHIC LATERAL SCLEROSIS AND FRONTOTEMPORAL DEMENTIA

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Keywords: C9ORF72, repeat expansion, polydipeptide, RNA foci

Expanded GGGGCC repeats in a non-coding region of the *C9orf72* gene are the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The pathogenic mechanism(s) caused by this expansion are not established, but may include loss of function from reduced expression of the *C9orf72* allele with the expansion and/or a toxicity derived from the expansion-containing RNA or its AUG-independent translation products. By production and analysis of multiple lines of transgenic mice expressing different lengths and levels of a repeat-containing human *C9orf72* gene, we now determine that *C9orf72* derived RNAs provoke a repeat length and expression level dependent accumulation of RNA foci containing sense or antisense repeat RNAs. As in human disease, *C9orf72* RNAs with repeat expansions drive AUG-independent translation of the encoded polydipeptides in brains and spinal cords. Like RNA foci, the accumulation of polydipeptides is shown to be dependent on both repeat length and expression level, with polydipeptide aggregates increasing with age. Transgenic mice expressing ~450 GGGGCC repeats develop increased anxiety and disrupted cognitive function by 12 months of age. In contrast, chronic reduction or complete elimination of *C9orf72* expression is shown to be well tolerated in mice without provoking ALS or FTD-like disease, at least up to 10 months of age. Our results identify one component of pathogenesis from *C9orf72* repeat expansion underlying ALS/FTD to be a gain of toxicity. Furthermore, our mice provide an *in vivo* platform for developing and validating antisense oligonucleotide therapy targeting repeat-containing *C9orf72* RNAs.

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C22 AN *IN VIVO* MODEL OF BMAA-INDUCED PROTEIN INCLUSIONS OF GUAM ALS/PDC

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Keywords: Guam, ALS/PDC, BMAA

Background: Amyotrophic lateral sclerosis/Parkinsonism Dementia Complex (ALS/PDC) is a neurodegenerative disease suffered by the Chamorro people of Guam. BMAA (β-N-methylamino-L-alanine), a non-protein amino acid produced by cyanobacteria (1), occurs in traditional dietary items consumed by the Chamorros. BMAA can be misincorporated in place of L-serine in proteins, causing protein misfolding and aggregation (2, 3). BMAA administered intravenously in mice rapidly crosses the blood brain barrier (4, 5). In rats, prolonged (30 day) intrathecal infusion of BMAA produces neurodegeneration (6). Although acute neurotoxicity was

previously observed in macaques fed BMAA (7), the consequences of chronic exposures are unknown.

Objectives: To determine if chronic dietary exposure to BMAA in non-human primates triggers neuronal protein inclusions similar to ALS/PDC, and if coadministration of L-serine can reduce protein inclusions.

Methods: Vervets *(Chlorocebus sabaeus*) in St. Kitts were given fruit dosed with BMAA for 140 days. One cohort of four vervets received BMAA, a second cohort received L-serine, a third cohort received L-serine plus BMAA, and a control cohort received rice flour. This experiment was subsequently replicated with three cohorts of eight adult vervets: one cohort was fed fruit dosed with BMAA, a second cohort received BMAA plus L-serine, and a third cohort received rice flour.

Results: All vervets fed BMAA developed neuronal protein inclusions similar to ALS/PDC. Free and protein-bound BMAA was found in brain tissues similar to ALS/PDC (8, 9). Vervets fed BMAA plus L-serine had few protein inclusions.

Discussion and conclusions: This replicated experiment suggests that ALS/PDC can be triggered by chronic exposures to BMAA, and may shed light on suggested linkages between cyanobacterial exposures and ALS elsewhere (10, 11). L-serine is now being evaluated as a possible therapy for ALS in FDA-approved human clinical trials.

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Session 4B ALS Heterogeneity and Progression

C23 IMPROVING THE CLASSIFICATION OF ALS – CAN WE MAKE IT LOGICAL?

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Keywords: phenotype, classification, diagnosis

There is a great deal of inconsistency in the way ALS specialists describe the different clinical patterns seen, and a consequent confusion for patients, research programs and diagnostic coding systems. For example, many websites and text books of neurology state that there are five types of ALS, which are ALS, progressive muscular atrophy, progressive bulbar palsy, pseudobulbar palsy and primary lateral sclerosis. This is not even internally consistent since the distinction between the bulbar palsies and other groups is based on nervous system level (bulbar vs spinal) while the remaining differences are based on anatomical level (upper motor neuron, lower motor neuron or both). It is compounded further by the use of motor neuron disease as an umbrella term in some countries, and ALS as an umbrella term in others, while at the same time, ALS describes the clinical pattern of the motor syndrome and a diagnosis. A good example of the confusion that arises is the El Escorial research diagnostic criteria. These are not in fact for the diagnosis of the motor syndrome, which can be made by a specialist with certainty even if the classification is "Possible", but for the certainty with which one can say the pattern is ALS as opposed to any other clinical pattern. ALS specialists also tend to use their own classifications, many of which overlap or can be understood by other specialists, but are not consistent between centres or individuals.

We now understand far more about the clinical profile of ALS than ever before, including the involvement of non-motor systems such as cognition, the natural history of different clinical presentations, and so-called deep phenotypes such as eye movement abnormalities. The time has come for a formal, unified, logical classification.

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C24 HOW COMMON ARE ALS PLATEAUS AND REVERSALS?

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Keywords: prognosis, progression, ALSFRS

Objectives: ALS plateaus and reversals are thought to be so uncommon that they are sometimes interpreted as evidence of a treatment effect. In fact, the frequency of these has never been measured in a large population. We determined the frequency of ALS plateaus and reversals in the PRO-ACT database.

Methods: Our teams independently reviewed longitudinal ALSFRS and ALSFRS-R data from PRO-ACT participants. The frequencies of participants experiencing plateaus (periods where scores did not change) were calculated over 6-, 12- and 18-month epochs. The percentage of participants ever experiencing plateaus and reversals (periods where scores improved) of different lengths were also calculated and plotted.

Results: Over 6 months, 25% of 3132 participants did not decline. Over 12 months, 16% of 2105 participants did not decline. Over 18 months, 7% of 1218 participants did not decline. Small ALS reversals were also common, especially over shorter follow up intervals; 16% of 1383 participants had a 165-day interval where their ALSFRS-R slope was greater than zero, and 14% of 1343 participants had a 180-day interval where their ALSFRS-R slope was greater than zero. Fewer than 1% of participants ever experienced improvements of 4 or more ALSFRS-R points lasting at least 12 months. Plateaus and reversals occurred at similar frequencies in separate analyses limited to placebo-treated PRO-ACT participants, confirming that these occurred independent of treatment assignment.

Conclusions: ALS plateaus and small reversals are common, especially over brief intervals. In light of these data, stable disease, especially for a short period of time, should not be interpreted as an ALS treatment effect. Large sustained ALS reversals on the other hand are rare, potentially important, and warrant further study. There are two main limitations to our study. First, the demographics of participants in ALS trials (and thus those in PRO-ACT) differ from non-participating patients in clinics. It is thus not known how generalizable our results are to patients outside of trials. Second, we measured ALS progression using the ALSFRS and ALSFRS-R. While these are the most commonly used measures of disease progression in ALS clinics and in current trials, they are unlikely to be perfectly sensitive to motor neuron loss. More sensitive ALS biomarkers are actively being sought and will eventually further illuminate the course of ALS progression.

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C25 PREDICTION OF SURVIVAL IN INDIVIDUAL ALS PATIENTS: INTEGRATION OF CLINICAL, COGNITIVE, GENETIC AND IMAGING DATA

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Keywords: survival, prediction, imaging

Background: Survival of patients with amyotrophic lateral sclerosis (ALS) is highly variable and prediction of survival of individual ALS patients is currently largely unknown, although many factors are known to be associated with survival on group level (1). This gap in knowledge of predicting factors hampers individual risk-assessment, stratification of patients for trials, and timing of clinical interventions. This results in increased uncertainty for patients, physicians and scientists and slows down development of new therapies.

Objective: To predict survival of individual ALS patients at time of diagnosis using clinical, cognitive, genetic and imaging data.

Methods: The dataset consists of 1936 population based ALS patients that gave informed consent for a Dutch ongoing study, the Prospective ALS study the Netherlands. Based on previous studies in ALS, 16 different variables were eligible for entry in the prediction model: gender, site of onset, age at onset, El Escorial criteria, diagnostic delay, forced vital capacity, slope of the ALS functional rating scale (ALSFRS slope), body mass index, past or current smoking, pack years, presence of frontotemporal dementia, verbal fluency index, frontal assessment battery total score, ALSFTD questionnaire score, *C9orf72* repeat expansion, UNC13A risk allelle (C/C). This multivariate Cox model was developed using backward elimination and bootstrap validation. Predictive performance was described in terms of discrimination, ie the ability to differentiate between surviving and deceased patients, and calibration, ie the agreement between observed and predicted risk, using respectively receiver operator curves and calibration plots. Predictive performance was reassessed with addition of imaging markers to the predictive model in 210 ALS patients.

Results: The prediction model consists of site of onset (hazard ratio (HR) 1.21, p=0.006), age at onset (HR 1.03, p<0.001), definite ALS according to the El Escorial criteria (HR 1.22, p=0.017), diagnostic delay (0.97, p<0.001), forced vital capacity (HR 0.99, p=0.011), ALSFRS slope (HR 1.71, p<0.001), presence of frontotemporal dementia (HR 1.52, p=0.031), presence of a repeat expansion in the *C9orf72* gene (HR 1.36, p=0.004). Receiver operator curves showed an area under the curve of 87.2% (95% CI 85.6-88.9) at three years after onset. With a mean absolute error of 3.1%, calibration plots showed good agreement between predicted and observed risk. Cortical thickness of several frontotemporal regions was independently associated with survival on group level, but addition of imaging parameters did not improve prediction accuracy in individual patients.

Discussion and conclusion: 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 counselling, and future individualized trial design a step closer.

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C26 PROSPECTIVE, LONGITUDINAL STUDY COMPARING THREE OUTCOMES MEASURES

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Keywords: quantitative strength, outcomes measures, disease progression

A clear unmet challenge in ALS clinical research is the ability to screen potential therapeutic agents quickly. Using more sensitive and accurate outcome measures could improve clinical trial efficiency, requiring fewer subjects during a shorter time period. Quantitative strength measures accurately reflect disease progression in ALS. However, current methods are either very expensive and inconvenient, or are unable to accurately measure strength throughout the entire strength range.

We developed a new device, Accurate Test of Isometric Strength (ATLIS) that is easy to use and accurately tests strength of strong and weak muscle groups with high reliability. The maximal force of 12 muscle groups in the arms and legs are tested using fixed, wireless load cells and a high-back, adjustable chair. Raw scores are converted to percent of predicted normal to allow accurate comparisons between persons. Establishing

a disease progression rate for each individual will allow us to detect small but clinically relevant therapeutic effects.

The primary goal of this project was to determine whether ATLIS could be an effective outcomes measure for clinical trials in ALS. The specific aim of this study was to determine whether ATLIS provides more a reliable estimate of progression compared with two commonly-used ALS outcomes measures: the ALS Functional Rating Scale-Revised (ALSFRS-R), and vital capacity (VC). We hypothesized that ATLIS data would be less variable than ALSFRS-R and VC both among subjects and around each subject's regression slope relative to the mean slope of each measure among ALS patients in a prospective, longitudinal study.

One hundred participants with ALS (70 males, 30 females) with a mean age of 57.8 years were enrolled in this study at five sites throughout USA. A total of 343 visits were performed. The 63 participants who had complete data from at least three visits followed over four to 21 months were included in the analysis.

The among-subject relative standard deviations for the arm and leg ATLIS scores (0.63 and 0.72, respectively) were substantially smaller than estimates for ALSFRS-R or VC (0.78 and 0.86 respectively). Within-subject or residual relative standard deviations of ATLIS arm scores (2.04) were also lower than alternative measures (ALSFRS-R: 3.09; VC: 4.96), but not leg scores (3.54). This supports the hypothesis that ATLIS provides a more reliable estimate of change compared to ALSFRS-R or VC and would decrease sample size requirements in future trials. Additionally, the mean ATLIS scores of subjects rated as normal for arm or leg function on the ALSFRS-R were 10 to 20 percent below matched norms suggesting that ATLIS may detect pre-clinical muscle weakness. Lastly, ATLIS may offer important predictive information for planning clinical care.

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C27 SLOW VITAL CAPACITY AND FORCED VITAL CAPACITY IN ALS – THE SAME REALITY?

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Keywords: slow vital capacity, forced vital capacity, respiratory involvement

Background/Objectives: Respiratory Insufficiency (RI) and its complications are the main cause of death in ALS. A comprehensive respiratory evaluation is fundamental at first visit and on follow-up. Slow (SVC) and forced (FVC) vital capacities are frequently used interchangebly but FVC seems to decrease more when obstructive involvement is present. In ALS there are no studies comparing SVC and FVC, the aim of this study.

Methods: Consecutive patients with probable/definite ALS (revised El Escorial criteria), followed since 2001 in our Unit and evaluated with: SVC, FVC, revised ALS Functional Rating Scale (ALSFRS-R); respiratory subscore of ALSFRS-R (RofALSFRS-R). Independent *t*-test compared SVC, FVC, ALSFRS-R, RofALSFRS-R in addition to maximal inspiratory (MIP) and expiratory (MEP) pressures, mouth occlusion pressure (P0.1), upper (ALSFRSul) and lower limb (ALSFRSll), ALSFRS-R subscores. Correlations between parameters were done using Person's correlation. Linear regression assessed SVC and FVC dependency on other parameters. Parameters were compared between patients with RofALSFRS-R >10 (G1 group) and <10 (G2 group) and correlations were reassessed.

Results: From the 500 patients included (onset age 62.3 ± 11.7 years; disease duration 14.7 ± 14.3; 271 men), onset form was spinal in 319, bulbar in 157, respiratory in 9 and axial in 15. FVC and SVC showed strong positive correlations in the all population and onset subgroups, and with MIP, MEP, ALSFRS-R, RofALSFRS-R ($p<0.001$) for the all population and for spinal and bulbar patients. Negative significant correlations were found with P0.1 ($p=0.016$) only in bulbar subgroup. In axial and respiratory subgroups, SVC or FVC did not correlate with other parameters except ALSFRSll in the axial subgroup ($p=0.042$ for SVC; $p=0.029$ for FVC) and MEP in respiratory patients ($p=0.019$ for SVC; $p=0.032$ for FVC). For all patients, SVC and FVC were dependent on gender, onset form, MIP, MEP, P0.1, ALSFRSb and ALSFRSll, RofALSFRS-R, but not on ALSFRS-R, onset age or disease duration. When comparing the 412 ALS patients in G1 to the 88 in G2, all parameters were significantly lower in G2 ($p<0.001$) except for disease duration and P0.1. SVC and FVC were correlated to each other in G1 and G2 ($p<0.001$).

Discussion and conclusion: This is the first ALS study comparing SVC to FVC and correlating them to each other and other respiratory parameters, including RofALSFRS-R. FVC is technically more difficult to perform if orofacial paresis is present. It can be lower in the presence of air trapping due to expiratory muscle involvement and athelectasis. Our study demonstrates that respiratory function test parameters and RofALSFRS-R are strongly correlated and present lower significant values when there is RI. Strong SVC and FVC correlations exist even when there is RI and show dependency on the same variables. This study has clinical as well as research implications as respiratory parameters are frequently study endpoints.

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Session 5A Gene Therapy

C28 TARGETING RNA MIS-SPLICING IN SMA

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Keywords: SMA, splicing, ISIS-SMNRx

Spinal Muscular Atrophy (SMA) is a motor-neuron disease, caused by loss-of-function mutations in the Survival motor neuron 1 (SMN1) gene. Patients retain one or more copies of the nearly identical, but splicing-defective SMN2 gene; SMN2 mainly expresses mRNA lacking exon 7 (the penultimate exon), resulting in an unstable protein isoform. The small amount of full-length mRNA and protein expressed from SMN2 is essential for survival of SMA patients, but only partially compensates for the loss of SMN1. Together with Isis Pharmaceuticals, we developed ISIS-SMN_{Rx}, an antisense oligonucleotide (ASO) complementary to a region comprising a potent splicing silencer in SMN2 intron 7 (1). This ASO efficiently promotes exon 7 inclusion and restores SMN protein levels in various tissues of SMA mouse models (2-5). I will present our data comparing CNS versus systemic delivery of ISIS-SMN_{Rx} in a severe SMA mouse model (4-6). Unexpectedly, our results indicate that SMA is not motor-neuron cell-autonomous, and suggest that correction of SMN2 splicing in peripheral tissues is necessary and perhaps sufficient for full phenotypic rescue, at least in the context of the mouse model. I will also summarize the ongoing clinical trials of ISIS-SMN_{Rx}.

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C29 DEVELOPING THERAPEUTICS FOR NEUROMUSCULAR DISEASE: FROM BASIC TO TRANSLATIONAL STUDIES

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Keywords: AAV gene therapy, SMA

Neuromuscular disorders are severely debilitating diseases with diseases such as Spinal Muscular Atrophy and Amyotrophic Lateral Sclerosis leading to death. Studies have implicated the cell autonomous and non-cell autonomous nature of these diseases. In this presentation, we will present the latest innovations in understanding disease mechanisms and will highlight the power of utilizing patient specific stem cells in order to model disease in a dish. Further, we will highlight the exciting results in the gene therapeutics space that have resulted in human clinical trials that are currently ongoing in Muscular Dystrophy and Spinal Muscular Atrophy as well as pre-clinical work in Amyotrophic Lateral Sclerosis.

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C30 A NEW AAV-BASED GENE THERAPY APPROACH FOR SOD1-LINKED ALS

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Keywords: AAV, SOD1 suppression, mouse model

Background: The suppression of mutant SOD1 protein in affected tissues of SOD1-linked ALS patients represents a promising therapeutic solution, based on the toxic gain of function acquired by the mutant enzyme. Therapeutic proof of concept of this strategy has been demonstrated in ALS animal models using either antisense oligonucleotides (1) or Adeno Associated Virus (AAV)-induced RNA interference (2,3). However, the rescue reported in these studies was not complete and the development of new approaches is necessary to increase the therapeutic efficacy of SOD1-gene silencing.

Objectives: Our objective was to provide an innovative gene therapy strategy for inducing strong and durable

human SOD1 (hSOD1) downregulation in the whole body of $SOD1^{G93A}$ mice treated either at birth or at the adult age.

Methods: We developed a new SOD1 silencing strategy by inducing splicing redirection of hSOD1-pre-mRNA (exon skipping) using antisense sequences (AS) targeting hSOD1 pre-mRNA. This generated a premature stop codon, thereby producing an aberrant transcript and activation of non-sense mediated decay (NMD). The silencing AS (sil.hSOD1) were delivered to both the CNS and the peripheral organs by co-injection of AAV10 vectors into the bloodstream (IV) and the lateral brain ventricles (intracerebroventricular, ICV) of newborn (P1) and adult $SOD1^{G93A}$ mice (P50).

Results: AAV10-sil.hSOD1 injection in neonatal $SOD1^{G93A}$ mice mediated a highly efficient hSOD1 exon skipping in the spinal cord, with 75% and 70% reduction of hSOD1 mRNA and protein levels, respectively, compared to control injected mice (AAV-CTR). The survival of the AAV10-sil.hSOD1 injected $SOD1^{G93A}$ mice was nearly doubled, with an increase of the mean life expectancy of 92% compared to AAV10-CTR injected mice (236 ± 11 *vs* 123 ± 1.6 days, $P < 0.0001$). AAV10-sil.hSOD1 delivery also delayed disease onset by 13 weeks compared to AAV10-CTR injected mice.

Interestingly, this approach was also highly efficient for rescuing adult $SOD1^{G93A}$ mice injected at 50 days, with a 58% of increase in life expectancy and a 75-days delay in disease onset, compared to control mice. AAV10-sil.hSOD1 delivery in adult mice also prevented the body weight loss phenotype and preserved motor function as well as skeletal muscle force over the study period. AAV10-sil.hSOD1 also protected motor neurons from ALS-induced degeneration and reduced the inflammatory reaction in the whole spinal cord.

Discussion and conclusions: Taken together, these results showed that AAV10-mediated exon skipping of hSOD1 mRNA induced strong, widespread and sustained reduction of SOD1 levels in the whole body of $SOD1^{G93A}$ mice injected at both pre-symptomatic and early-symptomatic stages, leading to the largest extent of life expectancy obtained to date. This strategy shows great potential and value for delaying disease progression, increasing life expectancy and improving the quality of life of SOD1-linked ALS patients.

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Session 5B Genetic Counselling

C31 ALS GENETICS: HELPING YOUR PATIENTS AND FAMILIES UNDERSTAND

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This presentation will familiarize practitioners with concepts to consider in counseling patients and families about genetics of ALS, both sporadic and familial. It will review basic principles of inheritance, genetic terminology, and the genes currently implicated in ALS. Pros and cons of genetic testing of both patients and individuals at risk for inherited ALS will be presented. The particular challenges associated with testing in C9ORF72 families will be discussed. Brief case studies will illustrate how to evaluate the appropriateness of testing, interpretation of testing results, and the range of emotional responses that may occur in patients and at risk individuals when receiving testing results.

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C32 GENETIC COUNSELLING IN THE POST-GENOMIC WORLD: A CLINICIAN'S PERSPECTIVE

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Keywords: genetics, counselling, therapy

A first draft of the Human Genome Mapping Project was published in 2000. It took 15 years and cost $3.3 billion to sequence one person's entire genetic code. Now this can be done in 1 week for around $1,000. The revolution in affordable genomics is set to have a major impact for patients affected by ALS and their families in terms of defining genetic risk but it may soon also inform their therapeutic choices.

To date mutations in thirteen genes are known to cause dominantly-inherited adult-onset ALS (SOD1, C9ORF72, TARDBP, FUS, OPTN, VCP, UBQLN2, SQSTM1, PFN1, MATRIN3, CHCD5, TUBA4A and TBK1). In European populations they collectively account for ~60% of all familial and ~10% of sporadic ALS patients. Apart from SOD1 and FUS all of these genes lead to cytoplasmic TDP-43 aggregation, which appears to be the final common pathway. The cause of the disease in the remaining 45% of families and 90% of sporadic ALS patients is unknown but a genetic basis is strongly implicated even in the absence of a family history.

In this talk I will discuss our current practice for diagnostic and predictive gene testing, pre-implantation genetic diagnosis and the prospect of gene therapy. In 10 years time genetics, epigenetics and transcriptomics are likely to influence our approach to many aspects of clinical care.

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C33 PRE-SYMPTOMATIC ALS GENETIC COUNSELING AND TESTING: EXPERIENCE AND RECOMMENDATIONS

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Keywords: pre-symptomatic, counselling, genetic testing

Background: Remarkable advances in our understanding of the genetic contributions to ALS have sparked discussion and debate about whether genetic counselling should be offered to patients with ALS. A related, but quite distinct question is whether pre-symptomatic genetic testing should be offered to family members who may be at risk for developing ALS. Existing guidelines for pre-symptomatic testing/counselling are mostly based on small number of subjects, clinical judgment and experience from other neurodegenerative disorders (PMID:21914052,18428003).

Objectives: To recommend updates to the guidelines for pre-symptomatic ALS genetic testing/counselling based on the evolving landscape of ALS genetics and our extensive experience from the *Pre-Symptomatic Familial ALS (Pre-fALS)* study.

Methods: *Pre-fALS* (now in its 8th year) is a prospective, systematic study of people at genetic risk for ALS but unaffected at the time of initial evaluation. During the enrolment process study participants elect whether or not to receive the results of genetic testing. Pre-test and post-test counselling is provided, by a single genetic counsellor, to participants selecting disclosure.

Results: As of May 2015, we have consented 249 unaffected first-degree relatives of ALS patients in whom the genetic cause of disease is known. Among the 194 who have completed initial screening, 26 had already undergone testing elsewhere; 34 chose not to receive results; and

134 (~70%) wished to receive results. Of the 134, 125 have completed genetic counselling and learned their results, with 47 (38%) found to be gene mutation carriers (*SOD1*, *C9orf72*, *FUS*, *TARDBP*, *VCP*).

Having conducted >260 pre-symptomatic counselling sessions (pre- and post-test), our approach has evolved over time. Moreover, this experience has enabled us to expand upon previously published recommendations for genetic counselling/testing in this population. For example:

- Prior to testing, individuals should be evaluated for psychosocial readiness, and testing deferred in the presence of an active psychiatric condition or the absence of an adequate social support system. Some long-term follow-up should also be in place to help minimize any potential adverse impact of learning the results.
- Counselling may be provided by phone for those who live afar (PMID:21285887).
- Special attention must be dedicated to communicating the limitations of currently available genetic testing and knowledge: what we know, what we do not know, and the implications of positive or negative test results in this context – for the individual being counselled and for their family members.

Discussion and conclusion: Pre-Symptomatic genetic testing with delivery of results can be done safely and effectively in the research arena. Experience from *Pre-fALS* highlights the real-world challenges of counseling people at genetic risk for ALS, thereby enabling us to validate some prior recommendations and refine others. Our experience-based recommendations are especially relevant given the growing interest in studying pre-symptomatic ALS.

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Session 6A Clinical-Pathological Correlates of Disease Progression

C34 UNDERSTANDING DISEASE PROGRESSION IN ALS

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Keywords: phenotypes, progressive muscular atrophy progression, respiratory failure

ALS disease progression represents a complex summation of pathogenic mechanisms that occur over space and time in the central nervous system. Disease progression is relatively orderly and predictable within an individual patient, but hugely variable between different patients. It has three primary determinants. The first is the focal body region of initial motor deficits, which are usually randomly located in bulbar, arm, trunk or leg regions. The second determinant is the distribution of the pathological burden between the upper motor neuron (UMN), and lower motor neuron (LMN) levels, which is highly variable. The degeneration often seems to spread outward along UMN and LMN anatomy. Importantly, the pathological burden is variably distributed and ranges from predominantly LMN, called progressive muscular atrophy (PMA), to predominantly UMN, called primary lateral sclerosis (PLS), and any combination in between. The third determinant of progression is progression rate, which reflects kinetics of motor neuron degeneration and is highly variable, ranging from slow to fast. Disease progression probably occurs independently at UMN and LMN levels. Patients with pathology that is relatively evenly distributed between UMN and LMN have overall progression that seems faster and more severe than patients whose disease burden is predominantly at one level. Because of the marked variability of these three determinants—site of onset, distribution of pathological burden between UMN and LMN, and rate of progression—ALS progression is highly heterogeneous and occurs on a vast continuum.

Progression of disease into the respiratory system, which is the primary predictor of survival and defines "end stage" disease, is a critical aspect of overall progression. Occasionally, respiratory weakness is the initial symptom, but more commonly it begins at some time in disease progression sooner or later compared to other body regions. The anatomy and pathophysiology of respiratory failure are poorly understood. Respiratory failure is rarely characterized in light of UMN and LMN pathologic anatomy. There are two distinct populations of LMNs innervating respiratory muscles: LMNs originating in the high cervical spinal cord innervate the diaphragm, and LMNs that originate in the thoracic spinal cord innervate intercostal and accessory muscles. UMN and supra-segmental control of LMNs are poorly understood.

A number of observational studies are now underway by several groups to characterize disease progression prospectively. These will be important for identifying biomarkers, and in re-defining clinical trial methodologies. Therapeutic strategies may be offensive, where the primary aim is disease control or disease eradication; or defensive, such as offered by stem cells, were delivery is regional and the aim is anatomical containment or local mitigation.

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C35 PRIONS IN NEURODEGENERATIVE DISEASES: AMYLOID STRUCTURES DICTATE DISEASE CHARACTERISTICS

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Keywords: tau, prion, amyloid

The prion model can explain many features of neurodegenerative diseases. This model predicts that accumulation of intracellular protein aggregates leads subsequently to their release into the extracellular space, where they are taken up by nearby cells, internalized, and seed further aggregation. This can account for phenotypic diversity and relentless propagation of neurodegeneration through brain networks. Our laboratory has elucidated the molecular mechanisms underlying propagation of tau protein aggregation. We created a sensitive and specific biosensor system to detect pathological seeds, and have observed that proteopathic seeding activity far precedes and predicts the development of classical tau neuropathology (1). Tau protein aggregates are taken up by direct binding to heparan sulfate proteoglycans on the cell surface (2). This triggers macropinocytosis and subsequent intracellular seeding. Interestingly, we have found that the minimal unit for spontaneous tau uptake and seeding is a trimer-identical to what has been reported for the prion protein (3). Tau aggregates exhibit the characteristics of prion strains in their ability to communicate specific patterns of pathology that can be passaged from cell to cell, or animal to animal by serial inoculation (4). With a simple cell propagation system we have isolated and characterized multiple tau strains. We have used this system to characterize strain composition in human brain samples from patients with a variety of tauopathies. We find considerable diversity. Some syndromes, eg Alzheimer's disease, are comprised of relatively homogeneous tau prion strains, while others, such as progressive supranuclear palsy, exhibit considerable diversity. This suggests that unique strains, or compositions of multiple strains, could underlie different tauopathy syndromes. To test this idea, we have inoculated multiple individual, well-characterized strains into transgenic mice. Each tau prion strain produces unique patterns of neuropathology and rates of spread within the brain. This work suggests that multiple neurodegenerative diseases associated with the accumulation of intracellular amyloids may be understood in light of

the prion model. This makes testable predictions about seed conformation and resultant neuropathology. Given the robust nature of tau prion propagation and faithful replication of defined amyloid structures, we propose that amyloids themselves encode the information to produce specific patterns of disease, and may also have a normal physiologic role.

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C36 FRONTOTEMPORAL DEMENTIA: ONSET, SPREAD, AND RELATIONSHIP TO ALS

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Keywords: neuroimaging, neuropathology, frontotemporal dementia

The anatomy of neurodegenerative disease can be understood in terms of two key aspects: onset and progression. Mechanisms controlling onset timing and location remain mysterious, and each disease features striking heterogeneity. Even in sporadic amyotrophic lateral sclerosis (ALS), onset may occur in upper or lower motor neurons, on either side, controlling limbs, trunk, mouth or face. Emerging data suggest that mechanisms underlying progression may be more tractable. Network analyses have revealed that each disease reflects degeneration of a specific large-scale network. Each vulnerable network, in turn, is anchored by a key "epicenter" whose functional-anatomical connections govern vulnerability of other regions, perhaps because prion-like corruptive templating begets trans-synaptic disease protein spread. In behavioral variant frontotemporal dementia (bvFTD), disease begins within a "salience network", anchored by the anterior cingulate and frontoinsular cortices, regions specialized for social-emotional-autonomic processing. BvFTD then spreads within that network before invading functionally related and connected systems. Patients with bvFTD due to TDP-43 proteinopathy often develop a staggered or simultaneous second site of onset in motor neurons, giving rise to an FTD-ALS picture. Likewise, patients with ALS may develop or begin with symptoms and signs of bvFTD.

What factors drive multifocal disease onset within the pyramidal skeletomotor and social-emotional systems? The most parsimonious explanation would be shared vulnerability mechanisms among neurons that reside only in and anchor the two systems. In the pyramidal motor system, corticospinal motor neurons reside in Layer 5b of the primary motor cortex, express transcription factors CTIP2 and FEZF2, and project large, long-range axons to the spinal cord anterior horn. In the social-emotional system, von Economo neurons (VENs) reside only in Layer 5b of the anterior cingulate and frontoinsular cortex, regions that may represent the major efferent and afferent representations of the viscero-autonomic-emotional system. VENs express CTIP2 and FEZF2, suggesting that they are long-range subcerebral projection neurons, perhaps with connections to brainstem autonomic integration centers or even spinal cord. By studying patients with bvFTD who die of MND we have demonstrated early selective vulnerability of VENs and related neurons to TDP-43 aggregation and degeneration. Shared vulnerability among VENs and motor neurons could explain the association between bvFTD and ALS, but mechanisms underlying this shared vulnerability remain unknown. Further work is needed to determine the normal transcriptional signatures of both pools, their points of intersection, and how these signatures go awry in the context of disease.

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C37 TDP-43 PROTEINOPATHIES: PATHOLOGICAL IDENTIFICATION OF BRAIN REGIONS DIFFERENTIATING CLINICAL PHENOTYPES

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Keywords: TDP-43 proteinopathies, simplified probabilistic statement, pathological identification of clinical phenotype

Background: The pathological sequestration of TAR DNA-binding protein 43 (TDP-43) into cytoplasmic pathological inclusions characterises the distinct clinical syndromes of amyotrophic lateral sclerosis and behavioural variant frontotemporal dementia, while also co-occurring in a proportion of patients with Alzheimer's disease, suggesting that the regional concentration of TDP-43 pathology has most relevance to specific clinical phenotypes. This has been reflected in the three different pathological staging schemes for TDP-43 pathology in these different clinical syndromes, with none of these staging schemes including a preclinical phase similar to that that has proven beneficial in other neurodegenerative diseases. To apply each of these three staging schemes for TDP-43 pathology, the clinical phenotype must be known

undermining the potential predictive value of the pathological examination.

Objective: The present study set out to test whether a more unified approach could accurately predict clinical phenotypes based solely on the regional presence and severity of TDP-43 pathology.

Methods: The selection of brain regions-of-interest was based on key regions routinely sampled for neuropathological assessment under current consensus criteria that have also been used in the three TDP-43 staging schemes. The severity of TDP-43 pathology in these regions-of-interest was assessed in four clinicopathological phenotypes: amyotrophic lateral sclerosis (n=27, 47-78 years, 15 males), behavioural variant frontotemporal dementia (n=15, 49-82 years, 7 males), Alzheimer's disease (n=26, 51-90 years, 11 males) and cognitively-normal elderly individuals (n=17, 80-103 years, 9 males).

Results: Our results demonstrate that the presence of TDP-43 in the hypoglossal nucleus discriminates patients with amyotrophic lateral sclerosis with an accuracy of 98%. The severity of TDP-43 deposited in the anterior cingulate cortex identifies patients with behavioural variant frontotemporal dementia with an accuracy of 99%.

Discussion and conclusion: This identification of regional pathology associated with distinct clinical phenotypes suggests key regions on which probabilistic pathological criteria, similar to those currently available for Alzheimer's disease and dementia with Lewy bodies, can be developed for TDP-43 proteinopathies. We propose and validate a simplified probabilistic statement that involves grading the presence of TDP-43 in the hypoglossal nucleus and the severity of TDP-43 in the anterior cingulate for the pathological identification of TDP-43 proteinopathy cases with clinical amyotrophic lateral sclerosis and behavioural variant frontotemporal dementia.

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C38 CORRELATION OF PATHOLOGICAL AND CLINICAL PHENOTYPES IN C9ORF72 CARRIERS

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Keywords: neuropathology, mRNA, C9orf72

Background: The *C9orf72* hexanucleotide expansion is the most common genetic cause of Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). It is characterised by phospho-TDP-43 pathology but also features specific to the disease, which include RNA foci and aggregations of dipeptide protein. Lower *C9orf72* mRNA levels and promoter and repeat methylation may also be of significance. The relative contribution of these pathologies to disease phenotype remains controversial.

Objective: To assess frequency of pTDP, foci and GA dipeptide and to quantify C9 gene expression and methylation in multiple brain areas with respect to clinical phenotype.

Methods: We obtained brain tissue from twelve patients with the *C9orf72* expansion, five with sporadic ALS and five controls without CNS disease from a clinically well-characterised cohort. Of the *C9orf72* patients, six were diagnosed with ALS, three with ALS-FTD and three with FTD. We examined paraffin embedded sections from five brain regions: non-motor frontal cortex, hippocampus, cerebellum, medulla, spinal cord. We characterised the distribution of p62, phospho-TDP and GA dipeptide pathology in neuronal cells and carried out fluorescence-in-situ-hybridisation for sense RNA foci. Furthermore, we extracted RNA and DNA from frozen samples of the same regions. RNA with RIN scores above 4 was reverse transcribed and the relative abundance of all three major *C9orf72* transcripts was quantified using TaqMan assays. DNA methylation of the repeat was quantified using the EpiMark kit.

Results: In our cohort, the brain areas affected by pTDP correlated best with the clinical diagnosis. GA dipeptide inclusions were rarely seen in hypoglossal nuclei or anterior horns of the spinal cord, and were ubiquitously expressed in the remaining areas studied. An increased frequency of GA dipeptide inclusions was observed in the hippocampus of FTD patients ($p < 0.05$). RNA foci were present in all brain areas studied and did not vary between phenotypes. In the C9 expansion carriers foci and dipeptide inclusions were more frequent in pTDP-43 positive cells ($p < 0.01$). Our qPCR showed the V2 transcript to be the dominant transcript. Levels of *C9orf72* V2 mRNA were highest in the cerebellum ($p < 0.05$). In C9 expansion carriers V2 mRNA levels were between 41 and 85% of control levels ($p < 0.01$). There was significant repeat methylation in *C9orf72* cases only in all areas but the cerebellum.

Conclusion: In our experimental setting, phospho-TDP43 pattern correlated better with clinical phenotype in *C9orf72* carriers than GA peptide and sense RNA foci. This emphasises the importance of pTDP-43 as a common pathway driving the ALS and FTD phenotypes in sporadic and *C9orf72* disease. A reduction of the V2 transcript in C9 expansion carriers compared to controls was seen in all areas, but cerebellar expression remained relatively high, correlating with lower repeat methylation and giving a potential explanation for the relative sparing of this region.

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Session 6B Respiratory Support

C39 LIVING AND DYING WITH INVASIVE HOME MECHANICAL VENTILATION IN PATIENTS WITH ADVANCED ALS: DECISION-MAKING, SURVIVAL AND WITHDRAWAL

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Keywords: survival, withdrawal, IHMV

Background: Patients suffering from Amyotrophic Lateral Sclerosis (ALS) have been offered non-invasive and invasive home mechanical ventilation (HMV) in West Denmark since about 1998. The use of HMV in ALS varies very much between centres and countries. Decision-making in care and treatment and advanced directives to withdraw are difficult tasks for patients and has to be made in participation with patients and relatives. Only a few studies address ethical aspects, survival and how to withdrawal treatment.

Objectives and methods: A Danish cohort of 409 ALS patients has been studied from 1998-2015 both qualitatively and quantitatively. Studies of decision-making, survival and withdrawal in patients with advanced stage ALS.

Results: Decision-making - In the decision-making process family always needs to be present and it is important to show patients and relatives how HMV functions in real life. In the dialogue with the patient and family health professionals need to address and talk about the patient's brain function and possible development of dementia.

Survival - Non invasive HMV (NIV) followed by invasive HMV (IHMV) is a possible treatment of respiratory symptoms in ALS and has a significant effect on survival. In relation to age, younger patients survived significantly better.

Withdrawal - Life prolonging treatment should be withdrawn in the event of IHMV patients developing Total Locked in Syndrome (TLS). Withdrawal of IHMV under deep sedation may be a medically, legally, and morally justified procedure to ensure a peaceful death in patients with advanced stage ALS and dependent on continuous IHMV.

Conclusion: Most important in decision-making to patients are family involvement and patients' autonomous decision-making. Patients live on average 56 months with IHMV. Patients make advanced directives to withdrawal of IHMV before start of treatment and withdrawal can be done legally and ethically correctly.

Acknowledgements: We want to thank all patients and staff at the Danish Centre for Home Mechanical Ventilation, Aarhus University Hospital, Denmark.

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C40 PERMANENT VENTILATION VIA TRACHEOSTOMY: A ONE-WAY STREET?

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Keywords: tracheostomy, weaning, ventilation

Ventilatory failure is the most common cause of death in patients with MND. For patients with limb-onset MND, non-invasive ventilation (NIV) improves symptoms, quality of life, and survival. With evidence of benefit, NIV use in the UK has increased significantly. For patients who fail to tolerate NIV (or in whom NIV is ineffective), then invasive ventilation via tracheostomy (TV) may be an option, although risks and benefits need to be considered very carefully. Existing data suggests that elective TV may be favoured for patients with bulbar-predominant disease who strive for survival; this may be especially evident in patients with young families.

Whilst TV may prolong survival, quality of life (QOL) is usually of greater concern for the majority of patients. In the absence of appropriate technical aids, TV results in significant communication difficulties and a greater loss of independence. For many, these factors outweigh the desire for survival. Patient-centred decision-making is crucial, but one also needs to be mindful that the significant burden of future care often relies in large part upon the carers. Finally, any wishes of the patient and care-giver(s) may be limited by the financial and practical capacity of the health care system. In an Italian series of patients, only 48% of patients admitted for TV were discharged home and median survival was less than one year. In most healthcare systems, elective TV therefore represents a second line option to NIV for a minority of patients.

Such decision-making is not always possible prior to an unanticipated deterioration that leads to acute invasive ventilation and intensive care unit (ICU) admission. Further, for a minority of patients, MND is diagnosed during an ICU admission. A chain of events may ensue that leads to prolonged invasive ventilation via tracheostomy (TV). In such circumstances, weaning from TV is considered to be difficult and prognosis poor; a recent meta-analysis assessing long-term outcomes after prolonged TV in ICU excluded patients with MND for this reason. Whilst continuation of TV may be appropriate for

some, for others it may result in an unacceptable QOL and an inability to return home.

The Respiratory Support and Sleep Centre (RSSC) at Papworth Hospital, Cambridge, UK provides a dedicated MND service that aims to optimise the respiratory care for patients with MND. In addition, we provide a specialist regional weaning service for patients requiring prolonged TV within ICU. This service aims to achieve discharge home with the maximum independence possible from TV. The aim of this presentation is to show that TV in patients with MND is not necessarily a one-way street.

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C41 THE DEVELOPMENT OF GUIDANCE FOR PROFESSIONALS IN THE UK FOR THE WITHDRAWAL OF ASSISTED VENTILATION AT THE REQUEST OF A PATIENT WITH MND

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Keywords: guidance, assisted ventilation, withdrawal

Background: A small number of ventilator-dependent MND patients request to have ventilatory support withdrawn. This situation may be ethically, practically and emotionally challenging for professionals (1,2). There are many examples of good practice, but some patients and family members related considerable gaps in support (3). Professionals also speak of the negative personal impact and identify a desire for the development of national guidance.

Objectives: To develop ethical and detailed practical guidance for withdrawal of ventilatory support at a patient's request, to be endorsed by the breadth of UK clinical professional organisations whose members regularly care for MND patients.

Methods: A multi-professional task group comprising primary care, palliative, respiratory, neurology, and intensive care physicians, plus respiratory care nurse specialists and a lay member. The group considered the recommendations arising from the exploration of experiences of 17 family and 50 professionals (3), and the published literature related to symptom management.

Results: The Guidance has 5 standards for care. 1) Patients should be made aware that they have the right to ask to stop assisted ventilation. They should be in no doubt that this has a legal basis and that healthcare professionals will support them; 2) A senior clinician should lead the planning and coordination of the withdrawal; 3) Withdrawal should be undertaken within a few days of an affirmed request from a patient with mental capacity; 4) Symptoms of breathlessness and distress should be anticipated and effectively managed; 5) Family members should have appropriate support and, with the patient's consent, opportunities to discuss the events with the professionals involved.

The Guidance acknowledges that more than one approach to symptom management may be effective, but all are underpinned by the principles of anticipatory sedation or augmented symptom management proportionate to the degree of ventilator-dependence.

The Guidance details the legal and ethical rationale, what to plan in advance, what to discuss with patients, families and colleagues and provisions for support for families after the patient has died.

The lack of a firm evidence base was identified and the Guidance includes request for submission of a core dataset to evaluate outcomes.

Discussion and conclusions: Professional organisations and their members have welcomed the development of this consensus-based Guidance which has gained from a collegiate sense of 'ownership' of the many challenges of this area of care.

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C42 MULTI-CENTER DIAPHRAGM PACING POST FDA APPROVAL STUDY ENROLLMENT COMPLETE: FAVORABLE PROCEDURAL SUCCESS, INTERIM SAFETY AND SURVIVAL FINDINGS

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Keywords: diaphragm pacing, respiratory failure, hypoventilation

Background: In 2011, DPS received FDA market approval for ALS under Humanitarian Device Exemption (HDE) based on a determination that the probable benefit to health outweighed the risk from its use. A post-approval study was required as a condition of approval.

Objectives: This study is a multi-center, open-label, evaluation to analyse data on DPS safety and survival benefit. Interim results are reported.

Methods: Patients with bilateral phrenic nerve function (BPNF) and chronic hypoventilation (CH) were eligible. Establishment of BPNF required: a phrenic nerve conduction study (PNCS) or diaphragm movement on a fluoroscopic sniff test. CH was documented by one or more of: FVC <50% of predicted; MIP <60cm H_2O; $PaCO_2 \geq 45$mmHg; or nocturnal SaO2 $\leq 88\%$. Implanted patients were evaluated for device and procedure related adverse events and survival (time to death or permanent tracheostomy mechanical ventilation (PTV)). Patients are followed every three months until they either meet the survival endpoint or 48 months.

Results: Sixty patients were enrolled at 11 centers. The mean time from ALS diagnosis was 465 ± 419 days. Stimulable diaphragm was determined by sniff test (61.7%) and PNCS (68.3%). Tests for CH were MIP (83.3%), FVC(13.3%), $PaCO_2$(5.0%), and SaO_2(6.7%). Concomitant treatments were NIV (70.0%), PEG (15.0%), cough assist (31.7%), and Riluzole (61.7%). Intraoperatively, 54 patients had confirmed stimulable diaphragm and were implanted. At 32 months since first enrollment, 32 patients are in follow-up and 22 patients have reached the survival endpoint. Median survival from implant is currently 20.9($\pm$ 2.4 SE) months. There was one probable procedure-related death from pulmonary embolism 8 days post-implant. Other adverse events included device malfunction that resulted in interruption/diminution of DPS therapy (n=8), percutaneous wire exit site infection (n=11), capnothorax requiring invasive intervention (n=3), perioperative complication which delayed the initiation of DPS therapy (n=2), and pneumothorax (n=1).

Discussion and conclusions: This study was designed to provide further data on safety and probable survival benefit of DPS. Enrollment of 60 patients was completed ahead of schedule in 24 months. Most were qualified on the basis of MIP, which has previously been shown to detect respiratory insufficiency earlier than FVC thus allowing earlier treatment (1). Ninety percent of patients had a stimulable diaphragm and had successful laparoscopic placement of the electrodes; this means 10% of patients had false-positive pre-operative analysis of intact diaphragm motor units for stimulation. Median survival of 20.9 months is consistent with 19.7 months in the US IDE pivotal study (2). Adverse events were consistent with previous reports with the most common being external electrode wire breaks and infections at the percutaneous wire exit sites. These events were resolved and patients continued therapy. The study has been amended to enroll additional patients.

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C43 A RANDOMISED CONTROLLED TRIAL OF THE EFFECTIVENESS OF THE NEURX RA/4 DIAPHRAGM PACING SYSTEM IN PATIENTS WITH RESPIRATORY MUSCLE WEAKNESS DUE TO AMYOTROPHIC LATERAL SCLEROSIS (ALS) (THE DIPALS TRIAL)

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Keywords: diaphragmatic pacing, clinical trials, respiratory failure

Background: Non-invasive ventilation (NIV) is part of the standard of care for the treatment of respiratory failure in amyotrophic lateral sclerosis (ALS), extending life by approximately 7 months. NIV however is not without its problems, particularly in patients with severe bulbar impairment. The NeuRx RA/4 diaphragm pacing system (DPS) stimulates the phrenic nerves near the motor end points within the diaphragm muscle, causing diaphragm contraction. Data from an uncontrolled multicentre cohort study led to FDA approval on humanitarian grounds of DPS for the treatment of respiratory failure in patients with ALS. We wished to establish by means of a randomised controlled trial, the efficacy and long-term safety of DPS when used in addition to NIV, compared to NIV alone.

Methods: DiPALS was a multicenter, non-blinded, randomised controlled trial undertaken at seven specialist ALS and respiratory centres in the UK. Eligible participants were aged 18 years or older, with a diagnosis of ALS laboratory supported probable, clinically probable or clinically definite according to the World Federation of Neurology revised El Escorial criteria; stabilised on

riluzole therapy for at least 30 days; and respiratory insufficiency by pre-defined criteria. The primary outcome was overall survival defined as the time from randomisation to death from any cause. DiPALS is registered at the ISRCTN Registry: ISRCTN53817913

Results: 74 participants (37 per arm) were randomised between Dec 5, 2011 and Dec 18, 2013. On December 18, 2013 the DMEC recommended suspension of recruitment having identified a concerning signal in the overall survival figures. Randomised participants continued as per protocol until June 2014 when the DMEC advised discontinuation of pacing in all patients. Follow up assessments continued until the planned end of the study in December 2014. The median survival was 11.1 months in the NIV plus pacing arm and 22.8 months in the NIV arm, with an adjusted hazard ratio of 2·27 (95% CI 1·22 to 4·25; p=0·01).

Discussion and conclusion: Diaphragmatic pacing should not be used as a routine treatment for patients with ALS in respiratory failure.

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Session 7A Genetics and Epigenetics

C44 DISTINCT BRAIN TRANSCRIPTOMES AND METHYLOMES IN C9ORF72-ASSOCIATED AND SPORADIC ALS

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Keywords: RNA misregulation, DNA methylation, RNAseq

Mutations associated with amyotrophic lateral sclerosis (ALS) account for ~20% of the overall ALS population. Thus, approximately 80% of cases are still genetically unexplained. Furthermore, genes associated with familial ALS are mostly unaltered in sporadic ALS (sALS), suggesting other disease culprits. Of interest, increasing evidence suggests that defective RNA processing and epigenetic regulation contribute to disease pathogenesis, this may be especially true for C9orf72-associated ALS (c9ALS). Specifically, RNA foci and dipeptide-repeat (DPR) proteins accumulating in c9ALS have the potential to modify RNA metabolism, and recent studies reported c9ALS-associated DNA and histone modifications, with hypermethylation of the *C9orf72* promoter and repeated region having the ability to modulate RNA foci and DPR protein toxicity.

To further explore RNA transcription and DNA methylation misregulation in ALS, we used next-generation sequencing to generate frontal cortices and cerebella transcriptome and methylome profiles of c9ALS and sALS cases. We compared data from those cases to non-disease controls. RNAseq data not only revealed altered expression, but also identified extensive alternative splicing (AS) and alternative polyadenylation (APA) defects in the these two brain regions, that were especially abundant in the cerebellum of both c9ALS and sporadic ALS cases. Co-expression network analyses of gene expression, and gene-association network analyses of AS data, revealed divergent molecular pathways associated with c9ALS and sALS.

To explore whether the RNA defects we identified could result from epigenetic modification, we used reduced resolution bisulfite sequencing (RRBS) to generate methylome profiles of c9ALS and sALS cases. Distinct methylome profiles were detected between c9ALS and sALS. Also, many of the 5mC events identified overlapped with genes found misregulated in the RNAseq data.

In conclusion, we demonstrated major transcriptome and epigenetic misregulation in ALS, especially in the cerebellum, and found divergent transcriptome and methylome profiles in c9ALS relative to sALS.

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C45 GENETIC OVERLAP BETWEEN AMYOTROPHIC LATERAL SCLEROSIS AND SCHIZOPHRENIA

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Keywords: pleiotropy, GWAS, schizophrenia

Background: Both sporadic and familial amyotrophic lateral sclerosis (ALS) are heritable, yet only a small proportion of cases can currently be explained by known genetic mutations. The largest genome-wide association studies (GWAS) to date have only confirmed the association of a small number of loci with ALS risk, leaving the remainder of the heritability of ALS weakly identifiable using common genetic variation. This contrasts with other traits such as schizophrenia, for which 108 loci have recently been implicated in a large GWAS, and for which common variation can explain as much as 22% of the variance in liability for disease. The availability of summary statistics for such GWAS provides the opportunity to conduct cross-trait studies of pleiotropy and genetic correlation, allowing potential to discover novel disease mechanisms and genetic risk factors. Prompted by the recent observation that pedigrees of ALS patients are enriched for neuropsychiatric disease and hypothesizing that schizophrenia has a neurodegenerative component, we used GWAS summary statistics to investigate the overlap between schizophrenia and ALS.

Objective: To determine the genetic correlation between schizophrenia and ALS.

Methods and results: Using linkage disequilibrium score regression, we replicated estimates for the SNP-based heritability of ALS (8.2%) and we estimated the genetic correlation between ALS and schizophrenia captured by common genetic variation to be 14.3%. This was supported by quantile-quantile plots for ALS summary statistics conditioned on schizophrenia p-values showing increased inflation in ALS statistics with increasing schizophrenia p-value threshold. This pleiotropic signal was further evidenced by polygenic risk scores based on common SNPs associated with schizophrenia, in which 0.3% of the variance can be explained by ALS case-control status. When other neuropsychiatric, neurodegenerative and anthropometric traits were considered, the genetic correlation with ALS was found to be specific to schizophrenia. Additional removal of duplicate controls between ALS and schizophrenia datasets will allow us to calculate per-SNP conditional false discovery rate for identification of shared genetic risk loci for both disorders. These findings have profound implications for our

understanding of the underlying biology of ALS as well as its classification and potential treatment.

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C46 LARGE SCALE GENETIC SCREENING IN SPORADIC ALS IDENTIFIES MODIFIERS IN C9ORF72 REPEAT CARRIERS

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Keywords: C9ORF72, oliogenic inheritance, repeat expansions

Sporadic ALS is considered to be a complex disease with multiple genetic risk factors contributing to the pathogenesis. Identification of genetic risk factors that co-occur frequently could provide relevant insight into underlying mechanisms of motor neuron degeneration. To dissect the genetic architecture of sporadic ALS we undertook a large sequencing study in 755 apparently sporadic ALS cases and 959 controls, analyzing ten ALS genes: *SOD1*, *C9orf72*, *TARDBP*, *FUS*, *ANG*, *CHMP2B*, *ATXN2*, *NIPA1*, *SMN1* and *UNC13A*.

We observed sporadic cases with multiple genetic risk variants in 4.1% compared to 1.3% in controls. This difference was not in excess of what is to be expected by chance (binomial test, $P = 0.59$). We did observe a higher frequency than expected of *C9orf72* repeat carriers with co-occurring susceptibility variants (*ATXN2*, *NIPA1*, *SMN1*; $P = 0.001$). Overall patient carrying mutations in multiple ALS genes had a younger onset and shorter survival, suggesting that having multiple mutations aggregates the phenotype. A striking number of *C9orf72* cases had a co-occurring *NIPA1*-repeat (P=0.006). Point mutations in *NIPA1* have been implicated in hereditary spastic paraparesis type 6, which is a disease characterized by progressive upper motor neuron signs. We also demonstrate that *NIPA1* repeat expansions are a risk factor for ALS independent of *C9orf72*.

Another intriguing observation is that our findings seem to imply that there a might be an increased propensity to develop repeat expansions in some ALS cases, as we observed multiple cases with repeat expansions in different genes (*ATXN2*, *C9orf72*, *NIPA1*).

Lastly we also observed two cases with a *C9orf72* repeat expansion combined with variants in two other genes (*C9orf72*, *ATXN2* and *UNC13A;* and *C9orf72*, *SMN1* and *UNC13A*). For both cases statistical analysis suggested that these combinations were not likely to be a chance finding with $P = 6.60 \times 10^{-4}$ and $P = 0.01$.

In conclusion we propose that phenotypic variability in *C9orf72* might be due to additional genetic risk factors and potentially multiple repeat expansions.

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C47 EXOME SEQUENCING OF FAMILIAL ALS INDEX CASES IDENTIFIES A NOVEL ALS GENE THAT BINDS CALCIUM

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Keywords: calcium, mutation, exome sequencing

Background: Exome sequencing of familial ALS (FALS) index cases and multiple affected members of ALS kindreds has recently revealed novel genes via segregation studies (*PFN1, MATR3, VCP* and *CHCHD10*) (1-4) and also burden analysis (*TUBA4a* and *TBK1*) (5-7).

Objectives: As part of a consortium effort, we analyzed 403 index FALS exomes to identify common founder non-synonymous variants present in multiple unrelated index cases to identify candidate ALS genes.

Methods: The exomes of 403 FALS index cases without mutations in known ALS genes were analyzed in an unbiased fashion. Successive filtering steps were used to identify single nucleotide changes present in 2 or more FALS cases but absent in the exomes of ~75,000 individuals in publicly available databases (1000 Genomes, NHLBI GO Exome Sequencing Project (ESP), UK 10K, ExAC, In-house UK exome controls, a cohort of Italian exome controls and UK local controls).

Results: Sixty novel heterozygous missense, nonsense or splice changing variants were shared by at least two index cases. As proof of methodology, the same approach was applied to 58 exomes with known mutations and identified 7 mutations in three known ALS genes (*SOD1, TARDBP or FUS*). The analysis of an independent replication cohort of 205 index FALS cases confirmed two of the novel variants in the discovery cohort and the total number of shared variants rose to 95. The top novel variant was a missense change found at the N-terminus of a gene encoding a calcium-binding protein (CBP), present in several affected members of two multi-generational British kindreds of the discovery cohort. The variant segregated with disease in both families, and was found in a third index FALS case of the replication cohort. Sequencing of 180 sporadic UK ALS cases identified the same missense variant. An 8 SNP and 2 microsatellite common haplotype was present in 5 variant carriers identifying a common founder. CBP carriers had a predominant bulbar presentation and late age of onset (average 72yrs). Additional novel or rare variants within CBP accounted for ~1.2% of FALS and ~1.5% of SALS, and 1.3% of total cases (n=10/788). Post mortem spinal cord tissue from the SALS harboring the common founder mutation showed classical pathological features of ALS with a unique feature of large CBP immunoreactive inclusions absent from controls. Transfection of primary motor neurons and HEK cells with CBP mutant constructs identified motif specific mislocalised, detergent resistant aggregates that were toxic to SH-SHSY cells. N-terminal CBP mutations resulted in a distinct lack of binding to another calcium binding protein of which the functional consequence is unknown.

Conclusion: Using a stringent filtering strategy of FALS exomes we have identified a non-synonymous missense change present in 4 cases that segregate with disease in 2 large UK families and have a common founder. The founder change results in gene specific aggregation in spinal cord post-mortem tissue and highlights the importance of calcium binding proteins in ALS.

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Session 7B Non-Motor Symptoms of ALS

C48 PSEUDOBULBAR AFFECT: FROM BIOLOGY TO MANAGEMENT

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Keywords: emotional expression, pathophysiology, neuroanatomy

Many patients with ALS suffer from bouts of crying or laughing in response to stimuli that would not have elicited such an emotional response before the onset of their disease. They may even have bouts of crying or laughing without any apparent motivating stimulus. This presentation will address the question of why this condition happens and how best to treat it. Pathophysiology of the condition and the neuroanatomy of emotional expression of laughter and crying in the human brain will be presented along with an unbiased overview of management strategies.

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C49 I CAN'T HELP THAT I LOOK SAD: THE EXPERIENCE OF EMOTIONAL LABILITY IN THE ALS PATIENT AND CAREGIVER

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Keywords: emotional lability, emotion regulation, caregiver burden

Background: There is increasing awareness that amyotrophic lateral sclerosis (ALS) symptomatology includes significant behavioural components. Emotional lability is a disabling symptom, whereby patients experience spontaneous or responsive uncontrollable emotional outbursts. Patient and caregiver reports of emotional lability, in particular, its association with patient mood symptomatology as well as caregiver burden have been little investigated.

Objectives: To investigate (i) the concordance between caregiver and patient ratings of emotional lability; (ii) the relationship between the presence of emotional lability with the patient's mood, emotion regulation abilities, cognitive function, and neuropsychiatric symptoms; (iii) the relationship between the presence of emotional lability and caregiver burden.

Methods: ALS patients without dementia (n=46) and their caregivers completed The Centre for Neurological Study – Lability Scale (CNS-LS), a 7-item questionnaire of emotional lability assessing the frequency of inappropriate tearfulness and laughter. Indices of the patient's mood (Depression Anxiety and Stress Scale), perceived ability to regulate emotions (The Difficulties in Emotion Regulation Scale) and caregiver burden (The Zarit Burden Scale) were also obtained.

Results: Patient (mean age=64; limb onset=67%; mean ALSFRS-R score=39) and caregiver ratings on the emotional lability scale were significantly correlated and demonstrated good concordance. Nearly half of all ALS patients (46%) scored above the cut-off (>13) on the emotional lability scale whereas this was seen in a third of caregiver ratings (31%). Patients report symptoms of emotional lability much more frequently than is recognized by the caregiver. Moreover, symptoms of tearfulness or laughter did not differ in patient ratings on the emotional lability scale although caregivers tended to report more frequent tearfulness than laughter ($p = 0.06$). Distinct patterns of correlations emerged in patient and caregiver ratings on the scale. Patient emotional lability scores correlated significantly with symptoms of anxiety (not depression) as well as with a perceived inability to control their own emotions. Caregiver (but not patient) ratings on the emotional lability scale correlated significantly with their own subjective experience of burden.

Discussion and conclusion: Patient and caregiver ratings on a brief emotional lability scale, while showing good concordance, highlight differences in their experience of emotional lability in ALS. First, patient ratings of emotional lability were independent of depression and associated instead with symptoms of anxiety as well as a perceived lack of emotional control. These findings suggest that the provision of emotion regulation strategies may be particularly beneficial for the patient. Next, the caregiver's experience of burden was directly associated only with their own (but not the patient's) ratings on the emotional lability scale. Furthermore, it is the combined use of patient and caregiver ratings on a brief emotional lability scale in the clinic that will facilitate understanding of the impact of emotional lability in ALS.

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C50 APATHY AND EXECUTIVE DYSFUNCTION IN ALS

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Keywords: apathy, initiation, cognition

Background: Research has shown specific cognitive deficits occur in ALS particularly in letter fluency and executive functions (1). Furthermore, one of the most frequently reported behavioural changes in ALS is apathy, however the association between these two cognitive and behavioural symptoms is unclear. We have previously demonstrated that ALS patients show increased Initiation apathy, a subtype of apathy characterised by reduced initiation of thoughts and action (2). An overlap between cognitive functioning and apathy subtypes has been proposed (3), indicating specific subtypes might associate with specific neuropsychological deficits.

Objective: To examine the relationship between cognitive deficits in ALS and specific apathy subtypes. Specifically our hypothesis is that ALS patients with increased Behavioural and Cognitive Initiation apathy will be more impaired than controls on executive tasks of cognitive initiation, namely fluency.

Method: To date 13 non-demented ALS patients (and carers) and 30 healthy age-education matched controls (and informants) were recruited. All completed the Geriatric Depression Scale (GDS), Apathy Evaluation Scale (AES) and Dimensional Apathy Scale (DAS; 4). The DAS was used to classify type of apathy. Patients and controls also completed the ECAS, and a neuropsychological battery containing tasks of planning and goal management, emotional perception (Ekman 60 Faces test), social cognition (Judgement of Preference) and intrinsic response generation (Verbal Fluency and Random Number Generation). Non-parametric statistics were used for behavioural and neuropsychological variable comparisons.

Results: 69% of ALS patients were classed as having Initiation apathy (based on self and carer DAS) with no controls scoring abnormally. Patients scored significantly higher than controls on both the self and informant/carer DAS Initiation subscale, self: U=60, p=0.000; informant/carer: U=58, p=0.000. No difference was found on the DAS Executive or Emotional subscales.

Patients performed significantly worse than controls on verbal fluency, U = 104, p = 0.012, Ekman 50 Faces test, U = 105.5, p = 0.018, time taken to plan in the planning task, U=118, p=0.042, and mean deviation time in the goal management task, U=99, p=0.011. Planned patient correlational analysis showed significant negative associations between the verbal fluency score and the Initiation apathy subscale, self: r(11)= –0.77, p<0.01. No such associations were found in controls.

Discussion and conclusion: Patients show increased Initiation apathy, which was associated with a verbal fluency deficit. These findings should be explored further to determine the extent of apathetic subtype cognitive deficits.

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C51 DIFFERENTIATING DEPRESSION FROM PBA-RELATED CRYING IN ALS: USE OF PHQ-9 AND CNS-LS

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Keywords: depression, pseudobulbar affect, psychometrics

Background: Diagnosis of depression in ALS is confounded by pseudobulbar affect (PBA) and motor deterioration, which may resemble emotional and somatic symptoms of depression, respectively.

Objective: To examine the validity of the Patient Health Questionnaire-9 (PHQ-9, a depression instrument based upon DSM-IV criteria) and the Center for Neurologic Study-Lability Scale (CNS-LS, an instrument to measure PBA) as measures of distinctive traits in ALS.

Methods: Multiple self-reported measures including PHQ-9, CNS-LS and a QOL measure EQ-5D™, were obtained from ALS patients using tablet devices in waiting rooms and a software system developed in-house at Cleveland Clinic (Knowledge Program). Analyses included descriptive statistics and exploratory factor analysis.

Results: Of 1008 ALS patients seen in the Cleveland Clinic Neuromuscular Center, 749 had simultaneous responses to the 9 questions of PHQ-9 and the 7 questions of CNS-LS. Cronbach's alpha was 0.83 and 0.87 for the two instruments respectively. Questions 3, 4 and 8 of PHQ-9 representing somatic symptoms disclosed a bimodal distribution and confounding by motor deterioration. However, near-perfect correlation (0.94) between the full PHQ-9 score and a subscore of non-somatic questions was found. Of EQ-5D dimensions, the fifth (Anxiety/Depression) correlated best with PHQ-9. From 693 patients who had also answered question 10 of PHQ-9, 113 (16.3%) met DSM-IV criteria for major depression. Corresponding ROC analysis indicated an optimal cutoff of 13 or higher of the PHQ-9 score. Exploratory factor analysis disclosed 3 latent factors underlying pooled questions of PHQ-9 and CNS-LS corresponding to depression, PBA (laughter) and PBA (crying). Moderate

correlation between PHQ-9 and the crying (but not the laughter) subscale of CNS-LS was seen.

Discussion and conclusions: PHQ-9 is an acceptable measure of depression in the ALS population. There is excellent discriminant validity between PHQ-9 and CNS-LS corresponding to the aims of these instruments.

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Session 7C Biomarkers

C52 ADVANCES IN PET IMAGING IN ALS AND RELATED CONDITIONS

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Keywords: imaging, PET, biomarkers

The lack of reliable biomarkers for diagnosis, prognosis, and response to therapy is a great unmet medical need in ALS. Positron emission tomography (PET) is an invaluable non-invasive imaging tool that provides a unique opportunity to study *in vivo* disease mechanism in patients and can serve as phamacodynamic biomarker for therapy development. Several PET tracers were tested in ALS and primary lateral sclerosis (PLS) adding to our fundamental understanding of disease mechanisms such as energy consumption (FDG), inflammation (TSPO), excitotoxicity (mGluR-5 and GABA), and oxidative stress (ATSM). This presentation provides an overview of the current landscape of PET imaging in ALS and PLS, and the future direction of this fast changing field.

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C53 METABOLIC SPATIAL CONNECTIVITY IN AMYOTROPHIC LATERAL SCLEROSIS: A 18-FDG PET STUDY

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Keywords: PET, spatial connectivity, discriminant analysis

Objectives: Positron emission tomography (PET) and volume of interest (VOI) analysis have recently shown in amyotrophic lateral sclerosis (ALS) an accuracy of 93% in separating patients from controls. The aim of the study was to disclose by spatial independent component analysis (ICA) the brain networks involved in ALS pathological processes and evaluate their discriminative value in separating patients from controls.

Methods: Two hundred fifty-nine ALS patients and forty age and sex-matched control subjects underwent brain 18F-2-fluoro-2-deoxy-D-glucose - PET (FDG-PET). Spatial ICA of the pre-processed FDG-PET images was performed. Intensity values were converted to z-scores and binary masks were used as data-driven VOIs. The accuracy of this classifier was tested versus a validated system processing intensity signals in 27 brain meta-VOIs. Support Vector Machine was independently applied to both datasets and 'leave-one-out' technique verified the general validity of results.

Results: The eight pathophysiological meaningful components discriminated patients from controls with 99.0% accuracy, being the discriminating value of bilateral cerebellum/midbrain alone 96.3%. Among the meta-VOIs right temporal lobe alone reached an accuracy of 93.7%.

Conclusions: Spatial ICA identified in a very large cohort of ALS patients spatially distinct networks showing a high discrimination value improving substantially the previously obtained accuracy. The cerebellar/midbrain component accounted for the highest accuracy in separating ALS patients from controls. Spatial ICA and multivariate analysis performed better in comparison to univariate semi-quantification methods in identifying the neurodegenerative features of ALS and pave the way for the inclusion of positron emission tomography in clinical trials and early diagnosis.

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C54 RETINAL THINNING IN AMYOTROPHIC LATERAL SCLEROSIS – A STUDY WITH OPTICAL COHERENCE TOMOGRAPHY AND DIFFUSION TENSOR IMAGING

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Keywords: retinal thinning, optical coherence tomography, biomarker

Background: Although motor neuron degeneration is the predominant feature in ALS, recent data point to a more widespread pathology comprising also non-motor regions. Retinal thinning has been reported in a variety of other neurodegenerative conditions, and a recent study reported cell involvement within the inner nuclear layer (INL) of the retina in two patients with *C9orf72* mutations (1).

Objective: We aimed to study retinal alterations in ALS using a systematic approach. We hypothesized that selective changes of specific retinal layers, as measured by Optical Coherence Tomography (OCT) may be a reflection of overall neurodegeneration. If so, retinal involvement may be regarded as a possible new biomarker in ALS.

Methods: Spectral domain OCT (Heidelberg Spectralis HD OCT) images were analyzed with an in house developed semi-automatic algorithm (2) to calculate the average thickness of single retinal layers in 73 ALS patients (51 spinal onset, 22 bulbar onset) and 20 matched controls. In a subgroup of 30 patients, additional Diffusion Tensor Imaging (DTI) data was acquired and the region of interest (ROI) based fractional anisotrophy (FA) was measured in the corticospinal tract (CST). Demographic data and the ALS-FRS score were collected for a correlation analysis.

Results: Multivariate regression analysis revealed a significant thinning of the INL (p=0.04) and the retinal nerve fibre layer (RNFL) (p=0.004) in patients compared to controls. Yet, subgroup analysis showed a significant difference only in spinal onset patients (INL: p=0.006; RNFL: p=0.002). Patients showed significantly reduced FA values of the CST compared to controls ($p<0.001$). We saw significant correlations between retinal thickness measurements and FA values of the CST in patients (p=0.005). There was no significant correlation between clinical parameters and retinal involvement.

Discussion and conclusions: Our study provides evidence for a retinal involvement with significant thinning in spinal onset ALS. Remarkably, the OCT measurements reproduce neuropathological findings of INL pathology and are in line with a recent study of retinal involvement in ALS (3). Interestingly, ALS patients show a statistically correlated reduction in FA and retinal thickness. We conclude that retinal involvement is in fact associated with neurodegeneration and may thus be regarded as a potential future technical marker in ALS. Further longitudinal studies in different patient populations and neuropathologic examinations of the retina in ALS will be needed in order to clarify the underlying pathological processes.

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C55 THE POTENTIAL OF WHOLE-BODY MUSCLE MR AS A BIOMARKER IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: MRI, muscle, biomarker

Background: An objective biomarker suitable for use in clinical trials is a major area of need in amyotrophic lateral sclerosis (ALS) (1), but has proven elusive, in part because of the clinical heterogeneity of disease onset and progression. Despite these challenges, ALS tends to be characterised by generalised muscle denervation. We hypothesised that whole-body magnetic resonance (MR) sampling of muscle in multiple body regions could be applied to account for clinical heterogeneity and quantify disease effects at the level of individual patients.

Objectives: In this preliminary report, we determine whether differences are detectable between ALS patients at the time of diagnosis and healthy controls using whole-body muscle MR methodology.

Methods: Fifteen ALS patients with varying patterns of disease onset and 20 healthy volunteers underwent whole-body muscle MR (Ingenia 3T, Philips, NL). Relative T2 signal estimates were extracted and averaged from muscles representing cranial, cervical, thoracic and lumbar segments.

Results: ALS patients demonstrated significantly higher T2 muscle signal ratios than controls (mean 0.66 arbitary units vs 0.50, p=0.0005).

Conclusions: Higher relative T2 signal intensity in ALS patients' muscles is consistent with denervation leading to increased proton fluidity or fat deposition. Whole-body muscle MR shows potential as a method of objectively quantifying denervation across different body regions and can capture ALS disease effects independently of clinical site of onset. The technique merits further investigation as a practical tool with which to objectively track disease changes in future clinical trials. The next step is to determine whether longitudinal changes are detectable within ALS patients.

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Session 8A Mechanisms of Intercellular Propagation

C56 TRANSLATIONAL PROFILING IDENTIFIES A CASCADE OF DAMAGE THAT INITIATES IN MOTOR NEURONS AND SPREADS TO GLIA IN MUTANT SOD1-MEDIATED ALS

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Keywords: SOD1, non-cell autonomous, RNA profiling

Background: Dominant mutations in the Cu/Zn superoxide dismutase (SOD1) gene account for 20% of the familial ALS. Ubiquitous expression of mutant SOD1 provokes non-cell autonomous toxicity and paralytic disease. Mutant protein in motor neurons accelerates disease onset, and mutant synthesis by neighboring glial cells accelerates disease progression. However, the molecular mechanism of how each cell type contributes to disease pathogenesis is not established.

Objectives: To determine cell type-specific and age-dependent damages induced by mutant SOD1 within motor neurons, astrocytes and oligodendrocytes during disease course.

Methods: We combine targeted Translating Ribosome Affinity Purification (TRAP) with high-throughput sequencing to identify translational profiling changes in spinal motor neurons, astrocytes and oligodendrocytes of mutant SOD1-expressing mice.

Results: Motor neurons are found to be most vulnerable to mis-folded SOD1 as a consequence of their naturally low level of expression of endoplasmic reticulum (ER) chaperones. Correspondingly, expression of mutant SOD1 initiates ER stress, synapse and metabolic abnormalities within motor neurons at disease onset. Induction of genes involved in inflammation and metabolism, including targets of PPAR and LXR nuclear receptors, are early changes within astrocytes. Dysregulation of myelination and lipid signaling pathways coupled with activation of ETS transcription factors are found within oligodendrocytes but only after disease onset. These data identify a temporal cascade of cell type selective damage initiating within motor neurons and propagating to oligodendrocytes that is essential to disease initiation and propagation.

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C57 PROPAGATION OF SOD1 MISFOLDING IN AN ALS MOUSE MODEL

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Keywords: SOD1, propagation, seeding

Background: One of the major unanswered questions in the study of ALS involves the symptoms, which appear to spread along neuroanatomical pathways to engulf the motor nervous system. Possible scenarios to explain these characteristics include non-propagative or propagative mechanisms. However, the overwhelming number of cases that present as a spreading paralysis indicates a strong predilection towards a propagative mechanism (1-3). This form of spread has been substantiated for prion diseases and has since been implicated in a number of other neurodegenerative diseases. We have recently developed an *in vivo* assay to induce MND and aggregate pathology in an SOD1 transgenic mouse line, which can now be used to further understand the pathogenesis of ALS (4).

Objectives: Using the indicated *in vivo* MND transmission model, we sought to address the possibility for SOD1 propagation throughout the CNS.

Methods: A spinal cord homogenate that contains abundant SOD1-YFP aggregates was prepared from a disease-induced G85R-SOD1:YFP mouse. This preparation was injected unilaterally into the sciatic nerves of heterozygous G85R-SOD1:YFP mice, which without inoculation, never develops YFP aggregate pathology or clinical disease. At multiple time-points post-inoculation, we sacrificed mice and analyzed tissue for the presence and spread of the induced YFP aggregate pathology.

Results: Although sparse, we first observed YFP aggregates in the ipsilateral dorsal root ganglia (DRG) and ipsilateral lumbar spinal cord. At 2 months post-inoculation, 2 of the 6 injected mice began to display signs of hindlimb paralysis in the injected limb. At this timepoint, YFP aggregates became more abundant in the ipsilateral lumbar spinal cord and DRG and began to appear on the contralateral side of these structures and also in more rostral levels of the spinal cord. Additionally, we began to see aggregates within the reticular formation of the brainstem. When we analyzed tissue from mice at the clinical stage of disease, widespread and abundant YFP pathology was observed in both the ipsilateral and contralateral DRG and within all levels of the spinal cord. The brainstem and midbrain were also observed to have numerous YFP aggregates.

Discussion and conclusions: Taken together, this data strongly suggests the ability for aggregates of SOD1 to propagate throughout the CNS following a focal accumulation of misfolded SOD1. Also, the temporal

accumulation of YFP pathology in this model implicates axonal transport as a means of spread.

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C58 USE OF A FLUORESCENTLY-TAGGED PERMISSIVE SUBSTRATE REPORTER SYSTEM TO DETECT SOD1 PRION-LIKE ACTIVITY IN LIVING CELLS: IMPLICATIONS FOR AN ALS THERAPEUTIC DRUG SCREEN

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Keywords: SOD1, propagation, drug screen

Background: Mutant SOD1 can confer its misfolding on wild-type SOD1 inside living cells (1); the propagation of misfolding can also be transmitted intercellularly (2). Our laboratory has previously reported that tryptophan (Trp) at position 32 of SOD1 is required for SOD1 self-recognition during prion-like conversion (1). A recent study has shown through crystal-structure analysis that 5-fluorouridine, a chemotherapy agent, binds at Trp32 (3). We therefore hypothesized that small molecules binding at or near the Trp32 site will block SOD1 template-directed misfolding. Through immunoprecipitation with misfolded SOD1-specific antibodies, we have shown that compounds such as 5-fluorouridine, 5-fluorouracil and uridine are efficient inhibitors of intercellular SOD1 propagated misfolding. We have now developed a robust reporter system that can detect the "prion-like" activity of misfolded SOD1 for use in a drug screen.

Methods: Fluorescently-tagged $SOD1^{G85R}$ has been previously shown to provide a substrate that is highly prone to misfolding and aggregation *in vivo* by a variety of templates (4, 5). We have generated a $SOD1^{G85R}$: GFP reporter construct that can be co-transfected into HEK293FT cells with propagation-competent mutant SOD1 templates. The number of induced $SOD1^{G85R}$: GFP aggregates is captured by confocal microscopy, and identified and quantified by image analysis. Up to 20,000 cells can be assessed per reading.

Results: Cells expressing $SOD1^{G85R}$:GFP reporter construct in the presence of a mutant $SOD1^{G127X}$ template show an average of 115 fluorescent aggregates, or particles/field, but only an average of 63 particles/field in its absence, and even less (35 particles/field) when Trp32 is mutated in the mutant template. Similarly, an average of 177 particles/field is observed in the presence of $SOD1^{G85R}$propagating template, but only an average of 24 particles/field is seen in the presence of $SOD1^{W32S,\ G85R}$.

Conclusions: We have developed a robust "prion activity" fluorescent reporter assay that can assess misfolded SOD1 propagation. The importance of SOD1 Trp32 has been validated via the assay and will therefore be adapted to screen small molecules predicted to bind the Trp32 pocket, and thus block propagated SOD1 misfolding.

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C59 TDP-43 PRIONOIDS TRIGGER ALS-ASSOCIATED PATHOLOGY IN NORMAL MOUSE BRAIN SLICES

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Keywords: TDP-43, prionoid, organotypic slice cultures

The RNA-binding protein TDP-43 aggregates and mislocalizes in the vast majority of sporadic amyotrophic lateral sclerosis (ALS), as well as approximately half of frontotemporal dementia (FTD) cases. Yet the role of TDP-43 aggregation in the pathogenesis of these diseases remains unknown. Moreover, understanding the molecular pathogenesis of ALS and FTD has been significantly hindered by the lack of reliable experimental models, which recapitulate TDP-43 pathology. In order to decipher the role of protein aggregation in ALS/FTD pathogenesis, we are mimicking the molecular events triggered by aggregation of ALS/FTD-linked proteins by inducing the misfolding and aggregation of their native forms, following a prion-like paradigm. We are building novel disease models to study ALS and FTD, and to identify mechanistic pathways driving their perpetuating progression.

Organotypic hippocortical slice cultures (HSC) from normal mice were kept stably in culture for up to three

months, allowing us to monitor the long-term effects of treatment with exogenous TDP-43 prionoids on endogenous TDP-43 and neuronal viability. Detergent resistant and structurally defined recombinant oligomers of TDP-43 were rapidly internalized by microglia and neurons of normal HSCs. This caused endogenous TDP-43 oligomerization in the cytoplasm, accompanied by pathologic posttranslational modifications, such as hyperphosphorylation. Pathologic TDP-43 alterations were persistent and intensified over time in HSCs even after the exogenous TDP-43 seeds were completely degraded by the microglia. Pathology progressed to TDP-43 nuclear clearance, which was associated with neuronal loss. Similar effects were observed on HSCs treated with stable TDP-43-RNA assemblies isolated from human ALS autopsies. Our data suggest that exogenous TDP-43 prionoids can induce TDP-43 subcellular mislocalization, hyperphosphorylation and neuronal loss in a well-defined timeframe that offers a window for molecular manipulations. This model will be extremely useful for determining the cascade of events that kills neurons in sporadic ALS and FTD and for screening potential therapeutic agents.

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C60 TDP-43 IS RELEASED IN ASSOCIATION WITH EXOSOMES

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Keywords: TDP-43, exosomes, protein aggregation

Background: Aberrant protein aggregates in neurons and glial cells are a pathological hallmark in neurodegenerations. The affected lesion with these aggregates is known to spread and this expansion is related to disease progressions. Previous reports suggest that the aggregated alpha-synuclein or tau itself can propagate in PD or AD, and exosomes contribute to this phenomenon, but it has not been elucidated in ALS/FTLD referred to as TDP-43 proteinopathies. In this study, we examined if TDP-43 is secreted in association with exosomes.

Method: We purified exosomes from Neuro2a cells or from frozen brains of mice or human autopsy cases using serial centrifugations and sucrose gradient fractionations and analyzed those exosomes by immunoblots and electron microscopy.

Results: Immunoblots showed that human WT, A315T, G348C, and 25kDa C-terminal fragment (25CTF) of TDP-43 was detected in exosomes, when they were overexpressed. There was no difference between WT and the two mutants TDP-43 expression in exosomes, but the amount of 25CTF TDP-43 was much higher than that of WT. TDP-43 was also detected in exosomes from hTDP-43-transfected Neuro2a cells by immunoelectron microscopy. The treatment of ethacrinic acid (EA), that induces TDP-43 aggregations, increased exosomal TDP-43 secretions in Neuro2a cells. Next, we evaluated exosomes of hTDP-43 A315T transgenic (mhTDP-43) mouse brains. TDP-43 in brain exosomes purified from 1-year-old mhTDP-43 mouse was increased compared with that of wild type mouse. Finally, brain exosomes of ALS patient were successfully purified, and it turned out that the quantity of TDP-43 in those exosomes were significantly larger than that of control patient brains.

Conclusion: Our data confirm that TDP-43 is released in exosomes from cultured cells and mouse/human brains, and that TDP-43 secretion is increased under the pathological conditions related with TDP-43 proteinopathy. Aberrant TDP-43 secretion in association with exosomes might underlie in the pathogenesis of TDP-43 proteinopathy.

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Session 8B Nutritional and Respiratory Assessment and Intervention

C61 WEIGHT LOSS AT DIAGNOSIS AND SURVIVAL IN ALS – A POPULATION-BASED STUDY

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Keywords: weight loss, ALS register, survival

Objective: We aimed in a population-based setting to (i) describe the nutritional status of ALS patients at time of diagnosis, (ii) evaluate the association between nutritional status and survival.

Methods: All patients recruited in the FRALim register (2000-2013) were considered to be included in this study. Socio-demographic and clinical data collected in the register database, were extracted. Time-to-death analyses were performed using multivariable Cox regression model. Model discrimination and calibration was assessed.

Results: Among 322 patients in the FRALim register, 261 patients (81%) were included. At the time of diagnosis, 50.6% of patients reported a weight loss (WL) as compared to usual weight of more than 5%: 14.6% with WL between 5 to 10% and 36.0% with a WL of more than 10%. WL at time of diagnosis was independently associated with survival (p=0.002). The risk of death was increased by 14% (5-23%) for each 5% loss of usual weight. Patients with a WL of 10% or more experienced a 45% increase in the risk of death (95%CI 6-99%) with respect to patients with a weight loss lower than 5% or no weight loss. The introduction of WL significantly improved the model's discrimination achieving a survival C-statistic of 79.5% (95%CI 75.6-83.5, p=0.006) at 12 months.

Conclusion: More than fifty per cent of ALS patients experience a weight loss at the time of diagnosis of more than 5%. This finding highlights the need of randomized trials to evaluate the effect of nutritional interventions using either gastrostomy, or oral nutritional supplements to improve ALS survivorship.

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C62 HYDRATION STATUS, DISEASE PROGRESSION AND SURVIVAL IN ALS PATIENTS

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Keywords: hydration, disease progression, survival

Background: Dehydration is a chronic concern in ALS patients; nevertheless, it is often overlooked in management (1) and its role in the progression of ALS has yet to be determined. Using doubly labeled water (DLW) and bioelectrical impedance spectroscopy (BIS) (2) in ALS patients we have created validated equations to accurately predict hydration status, specifically total body water (TBW) and water intake (WI). We hypothesize that suboptimal hydration predisposes to: (a) more rapid disease progression, and (b) decreased survival in patients.

Objectives:

(1) To use previously created equations for TBW and WI from a test cohort of ALS patients, in a validation cohort of patients (n=275):

Equation 1a: TBW (by DLW) = −1.809 − 6.255 (males) + 0.209 × weight (kg) + 0.140 × height (cm)

Equation 1b: TBW (by BIS) = −15.892 − 4.402 (males) + 0.274 × weight (kg) + 0.180 × height (cm)

Equation 2: Water Intake (L/d) = −1.119 + 0.022 × weight (kg) + 0.063 × ALSFRS-R

(2) To examine disease progression and survival in ALS patients based on hydration status

Methods: Data are being collected from a new cohort of 275 ALS patients evaluated in the National ALS Center clinic at the University of Vermont Medical Center. In addition to TBW and WI, disease progression, as measured by change in weight, ALSFRS-R and FVC, and survival, will be examined adjusting for known prognostic variables (3). The relationship between hydration status, as estimated by the three Equations above, and clinical measures of disease progression, will be examined by regression analysis. T-tests will also be used to compare mean disease progression rates for patients above and below the median of estimated TBW and WI, and using hydration as a continuous variable. Kaplan-Meier

statistics and proportional hazards regression will be used to assess the effect of hydration status on survival.

Results: We have previously shown in the cohort of 80 patients that survival in dehydrated patients is significantly shorter after adjusting for prognostic variables (2). From a sample of n=60 patients ("test set") studied with DLW, we observed correlation coefficients significant at $p < 0.05$ for all three regression equations given above. In n=20 patients ("validation set"), acceptable correlation coefficients were seen between the observed values and values predicted using the equations above. Kaplan Meier survival, adjusted for baseline prognostic variables, was significantly reduced in dehydrated patients (median survival 13 mos vs 25 mos, dehydrated vs non-dehydrated, p=0.05, log rank test). Results on rates of disease progression and survival will be presented from the cohort of 275 patients.

Discussion and conclusion: If our hypotheses are correct, results of this study will provide confirmatory evidence of hydration status influencing disease progression and survival in a validation cohort of patients, emphasizing the need for examining hydration to effectively manage ALS patients.

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C63 A RISK-STRATIFYING TOOL TO FACILITATE SAFE LATE-STAGE PERCUTANEOUS ENDOSCOPIC GASTROSTOMY IN MND

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Keywords: gastrostomy, FVC, risk

Background: Current guidelines warn against insertion of percutaneous endoscopic gastrostomy (PEG) in MND patients whose forced vital capacity (FVC) is less than 50% predicted, due to increased risk of procedure-related complications such as aspiration. Radiologically-inserted gastrostomy (RIG) is often suggested as an alternative, but in our experience the procedure requires the patient to be supine, which those with advanced disease typically cannot tolerate. RIG also requires high levels of analgesic sedation, which may place patients with respiratory insufficiency at higher risk of procedural complications.

Objectives: To review outcomes across a large series of PEGs in the Oxford MND Centre, including our introduction of a risk-stratification tool applied through a dedicated MND Centre Nutrition Clinic that adapts care in high-risk patients to allow PEG to be undertaken.

Methods: We identified 91 MND patients undergoing PEG insertion between the start of dedicated records in October 2011 and April 2015. Our risk-stratification tool was used to identify patients at high risk for sedation-related complications based on FVC, use of non-invasive ventilation and blood gas parameters. In such cases, PEG was performed in a semi-supine position, using a reduced sedative dose and in some cases nasal non-invasive ventilation.

Results: Twenty-one per cent of cases had a pre-procedure FVC$<50\%$ (overall cohort mean FVC=$65\% \pm 20$). The mean disease duration in patients with FVC$<50\%$ was 28 ± 32 months versus 26 ± 28 months for those with FVC $\geq 50\%$ (p=0.594), and ALSFRS 28 ± 7 versus 31 ± 6 respectively (p=0.128). Four deaths occurred within 30 days, and 13 within 6 months of procedure. The proportion of these deaths with pre-procedure FVC $<50\%$ was 4/4 (p=0.02) and 4/13 respectively (p=0.2). Across the whole cohort there were no occurrences of aspiration and no significant differences in other complications between pre-procedure FVC group.

In a sub-group of the patients who were risk-stratified prior to PEG insertion (29/91), there was one death at 30 days (p=0.7) and no significant difference in overall complications. The dose of midazolam administered to those deemed high-risk was lower (2.9 ± 1.2 mg versus 1.9 ± 0.3 mg, p=0.02), though there was no significant difference in fentanyl dose (57.8 ± 27.0μg versus 41.7 ± 12.5μg, p=0.187).

Conclusions: Our experience is that PEG can be safely undertaken in MND patients with FVC $<50\%$, and special attention to higher-risk cases through pre-screening is associated with a lower dose of benzodiazepine sedation. The apparently higher rate of death at 30 days seems more likely to reflect the natural history of the disease, rather than specific procedural complications.

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C64 DISCRIMINATE ABILITY OF THE EATING ASSESSMENT TOOL FOR PREDICTING ASPIRATION

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Keywords: dysphagia, screen, aspiration

Background: Oropharyngeal dysphagia is prevalent in individuals with amyotrophic lateral sclerosis (ALS); leading to malnutrition, aspiration pneumonia and death. These factors necessitate early detection of at risk patients to ensure maintenance of safe oral intake and

pulmonary function. The aim of this study was to determine the discriminant ability of the Eating Assessment Tool-10 (EAT-10) to predict airway safety status (aspiration) during swallowing in ALS.

Methods: 75 individuals with ALS (El-Escorial criteria) completed the EAT-10 survey and underwent a standardized videofluoroscopic evaluation of swallowing (VFES). Two blinded raters determined airway safety using the Penetration Aspiration Scale (PAS). ALS patients were divided into safe (PAS=1-2), penetrators (PAS=3-5) and aspirators (PAS=6-8). A between groups ANOVA was performed for and discriminant ability of the EAT-10 to detect aspiration assessed using receiver operator characteristic analysis.

Results: A significant main effect was observed for EAT-10 scores across airway safety groups ($F(2)=27.60$, $p<0.001$). Mean EAT-10 scores for safe, penetrators and aspirators (SEM) were: 4.2 (0.78) vs. 5.90 (1.96) vs. 20.50 (3.19) respectively. Post hoc analysis revealed that EAT-10 scores were higher (worse) in aspirators vs. safe swallowers and aspirators vs. penetrators ($p<0.001$). The EAT-10 demonstrated good discriminant ability to identifier aspirators (AUC=0.88). An EAT-10 cut point of 8 had a sensitivity of 85.7%, specificity of 71.9%, a negative predictive value of 95.5% and positive predictive value of 42.9%. An ALS patient was 3.05 times more likely to aspirate if they scored ≥ 8 on the EAT-10.

Conclusions: ALS patient self-reports of swallowing impairment using the EAT-10 was able to differentiate safe vs. unsafe swallowers. The EAT-10 screen could represent a quick and meaningful aide to dysphagia screening in busy ALS clinics for identification and referral of dysphagic individuals.

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C65 A RANDOMIZED SHAM CONTROL TRIAL OF EMST ON BULBAR FUNCTION

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Keywords: EMST, therapy, bulbar

Background: ALS patients are at increased risk for airway compromise due to dysphagia (leading to material entering the airway during swallowing) and dystussia (contributing to the inability to effectively eject tracheal aspirate). Sequelae of aspiration in ALS include malnutrition, compromised respiratory function and respiratory failure, pneumonia, increased risk of death and degraded quality of life and psychological health. We have recently reported that expiratory muscle strength training (EMST) is feasible, safe and lead to improvements in expiratory force generating pressures, swallowing kinematics, cough spirometry and airway protection during swallowing in a pilot study of 25 ALS patients. Further work is needed to validate these preliminary findings and to further elucidate the potential role of exercise in this patient population.

Objective: To determine the efficacy of a targeted bulbar strength training program (EMST) on maximum expiratory pressure (MEP), swallow kinematics, cough spirometry, quality of life and disease progression in ALS patients with mild to moderately severe symptoms.

Methods: 50 patients with mild-moderate ALS (possible, probable or definite Revised El-Escorial Criteria) participated in this randomized blinded sham-controlled clinical trial. Patients completed eight-weeks of daily training with an active (n=25) or sham (n=25) device at home and with weekly home therapy visits. The primary outcome variable was MEP (cmH_20). Secondary measures included: kinematic and temporal swallowing indices, cough spirometry measures and the Penetration Aspiration Scale score (an index of airway safety during swallowing). Tertiary outcomes include patient-reported measures of: swallow-related quality of life, dysphagia severity and functional oral intake using validated scales (SWAL-QOL, EAT-10, FOIS respectively). Finally, the impact of EMST on disease progression and MEPS was tracked over time with quarterly follow-up evaluations.

Discussion: At time of abstract submission, 46 individuals have completed this trial with the remaining four patients currently completing treatment with an anticipated completion date of June 24th. Interim data indicate a significant time by group interaction for the primary outcome variable, MEP ($F(1)=9.10$, $p=0.01$). Post-hoc analysis revealed a significant increase in MEPs for ALS patients in the active EMST group ($p=0.04$, mean difference $27.01cmH_20$) and a significant between groups difference (active vs. sham) at the post-treatment time point ($p=0.03$, mean difference $50.45cm\ H_20$). All data will be analyzed once the trial is completed within the next month.

Conclusion: Current interim data from this RCT confirm our previous findings and suggest that strength training of bulbar musculature may be beneficial for improving and maintaining expiratory generating pressures and may impact measures of global disease progression.

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Session 8C Electrophysiology and Imaging

C66 MUNIX DETECTS PRESYMPTOMATIC MOTOR UNIT LOSS IN ALS PATIENTS

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Keywords: MUNIX, pre-symptomatic, muscles

Background: MUNIX is a good candidate to serve as a biomarker in ALS. It reflects the number of functioning motor neurons (MU) in a given muscles. It has shown to strongly correlate with disease progression. However, it is not known whether MUNIX is capable of detecting MU loss in presymptomatic muscles.

Objective: To follow longitudinally MUNIX in APB, ADM, Biceps, AH, TA and EDB with Medical Research Council (MRC) Scale for Muscle Strength grade 5.

Methods: Data were taken from a longitudinal multicentre MUNIX study and decline rates of each muscle were calculated. All muscles from 64 subjects with at least 2 MUNIX measurements with MRC5 were accepted. To calculate the relative decline per month, linear mixed-effects models with month nested in muscles were performed. To compare the relative decline for MUNIX in each muscle, linear mixed-effects models with muscle nested in month were performed.

Results: Data from 164 individual muscles fulfilled the criteria for analysis. Average monthly decline rate was 0.94%/month for APB, 1.18% for ADM, 1.11% for biceps, 1.10% for TA, 1.76% for EDB and 1.10% for AH. Changes were increasingly significant when pooling all muscles after 6 months ($p<0.04$). Individual muscles except for biceps showed significant changes ($p<0.05$) at various points in time.

Conclusion: MUNIX is able to detect pre-symptomatic MU loss. It is assumed, that with an effective medication the decline rate in clinically strong muscles will also be reduced.

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C67 THE NATURAL HISTORY OF MOTOR CORTICAL FUNCTION IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: threshold tracking transcranial magnetic stimulation (TTTMS), intracortical inhibition, natural history

Background: Hyperexcitability of both upper (UMN) and lower motor neurons (LMN) potentially contributes to motor neuron degeneration in ALS, with dying forward hypothesis, UMN hyperexcitability inducing LMN hyperexcitability and degenerations. We revealed threshold tracking transcranial magnetic stimulation (TTTMS) detects motor cortical hyperexcitability in ALS, and this finding provides assistance for diagnosis (1). However, the natural history of motor cortical function in ALS has not been established.

Objectives: To disclose the relationship between disease stage and motor cortical function in ALS.

Methods: ALS patients (n = 189) without a family history or an inexcitable cortex were included. We performed univariate correlative and multivariate linear regression analyses using single pulse TMS parameters (resting motor threshold, motor evoked potential (MEP) amplitude, MEP latency, MEP/compound muscle action potential (CMAP) amplitude ratio, central motor conduction time and cortical silent period) and TTTMS parameters (short-interval intracortical inhibition (SICI) at 1ms, SICI at 3ms, averaged SICI (interstimulus interval 1-7ms) and intracortical facilitation). Disease stage was defined as: 1) Proportion of disease duration (only in deceased 105 patients), 2) Time from onset, 3) CMAP amplitude. In multivariate analyses, clinical factors which may affect TMS parameters were included with disease stage.

Results: Univariate and multivariate analyses, using proportion of disease duration, revealed SICI at 3ms ($p = 0.028$, $R = -0.22$ and $p = 0.048$, $\beta = -0.19$, respectively) and averaged SICI ($p = 0.034$, $R = -0.21$ and $p = 0.048$, β

= -0.21) decreased with disease progression, while these parameters did not demonstrate significant relationships with CMAP amplitude. Using time from onset, CMCT ($p = 0.010$, $R = -0.22$ and $p = 0.0022$, $\beta = 0.25$) was related to disease progression. As expected, utilizing CMAP amplitude, prolonged MEP latency ($p = 0.0039$, $R = -0.24$ and $p = 0.00096$, $\beta = -0.26$), deceased MEP amplitude ($p < 0.000001$, $R = 0.45$ and $p < 0.000001$, $\beta = 0.45$) and increased MEP/CMAP ratio ($p < 0.000049$, $R = -0.34$ and $p = 0.00014$, $\beta = -0.31$) were associated with CMAP decrease.

Discussion: SICI represents the function of cortical inhibitory interneurons, and in ALS, loss of inhibitory interneurons has been reported. Thus, our findings suggest inhibitory interneurons degenerate with disease progression, but this degeneration may not be parallel with loss of lower motor neurons. This may suggest that additional factors may contribute to LMN loss. Longitudinal observation of TTTMS may support diagnosis in patients with an unclear diagnosis.

Conclusion: Inhibitory interneuronal function measured via SICI decreases with disease progression.

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C68 RILUZOLE EXERTS SHORT-TERM EFFECTS ON CORTICAL HYPEREXCITABILITY IN SPORADIC ALS

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Keywords: riluzole, cortical hyperexcitability, neurophysiology

Objectives: Riluzole is the only neuroprotective agent to date shown to be effective in amyotrophic lateral sclerosis (ALS). The mechanism by which riluzole exerts neuroprotective effects in is via antagonism of glutamate, and recent studies in ALS patients have established partial normalization of cortical hyperexcitability. Given the modest therapeutic benefits, the duration of riluzole's effect in ALS is probably short, although this remains to be fully elucidated. Consequently, the present study assessed longitudinal effects of riluzole on cortical excitability in a cohort of sporadic ALS patients, by utilising threshold tracking transcranial magnetic stimulation (TTTMS).

Methods: Studies were longitudinally undertaken on 18 sporadic ALS patients, with cortical excitability assessed at baseline (prior to initiation of riluzole), at 4, 8, and 12 weeks post riluzole initiation. Motor evoked potenitals were recorded over the right abductor pollicis brevis muscle. Statistical analysis was undertaken with a Wilcoxson paired analysis, using a Bonferroni correction.

Results: At baseline, cortical hyperexcitability was evident in the sporadic ALS cohort, as indicated by a marked reduction in SICI ($SICI_{Baseline}$ 3.2%). Riluzole therapy resulted in a marked increase in SICI at 4 weeks ($SICI_{4\,weeks}$ 9.1% $P < 0.01$), which was maintained at 8 weeks ($SICI_{8\,weeks}$ 8.6%, P value compared to baseline $P < 0.01$). Interestingly, at 12 weeks, SICI again reduced to baseline values ($SICI_{12\,week}$ 3.24%, $P = 0.03$) despite riluzole therapy. There were no significant changes in other TTTMS parameters such as resting motor threshold; motor evoked potential amplitude or cortical silent period duration.

Conclusion: Our study suggests that the protective effect of Riluzole on cortical dysfunction may be short, peaking at 4-8 weeks post drug initiation. Thereafter, there is ongoing and progressive cortical dysfunction as measured by Threshold Tracking TMS studies. This is an important finding as it gives us neurophysiological insights into the duration of effect of Riluzole. Clinically it is known that Riluzole only prolongs survival by a few months. Utilising our TMS studies we have shown that the neurophysiological effect of Riluzole is consistent to the clinical findings, hence there is an unmet need to identify more potent novel therapeutic agents to modify disease progression in ALS.

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C69 EFFECTS OF THE C9ORF72 REPEAT EXPANSION: A NEUROIMAGING INVESTIGATION OF BRAIN MORPHOLOGICAL CHANGES IN ASYMPTOMATIC MUTATION CARRIERS

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Keywords: C9orf72, imaging, asymptomatic carrier

Background: The *C9orf72* repeat expansion is an important cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Recent neuroimaging studies ALS patients with a *C9orf72* genotype have found widespread cerebral abnormalities, with structural involvement extending to frontal and temporal regions (1). An unanswered question is whether any of these brain

changes are already present in *C9orf72* carriers, before motor or cognitive signs become clinically apparent. Asymptomatic mutation carriers provide a unique opportunity to address this question.

Objectives: To study possible affects of the *C9orf72* repeat expansion on brain morphology in asymptomatic mutation carriers, prior to disease onset.

Methods: In total, 39 healthy family members from a large family with a history of ALS were included. Neurological examination was performed and cognitive performance was assessed in all family members. Genetic analysis identified 16 asymptomatic carriers of the repeat expansion and 23 non-carriers. Aiming to diminish the effects of genetic variation between subjects, apart from the *C9orf72* repeat expansion, brain morphology of carriers was compared to the non-carriers. Cortical thickness, subcortical volumes and white matter connectivity, as assessed from high resolution T1-weighted and diffusion-weighted magnetic resonance images, were evaluated. For comparison, 14 *C9orf72* ALS patients and 28 healthy unrelated controls were included.

Results: We found temporal, parietal and occipital regions to be thinner ($p < 0.05$) and the left caudate and putamen to be smaller ($p < 0.05$) in asymptomatic carriers compared to non-carriers. Cortical thinning of the primary motor cortex and decreased connectivity of white matter pathways (global, corticospinal tract and corpus callosum) were observed in *C9orf72* ALS patients, but not in asymptomatic carriers.

Discussion: The present findings provide a rationale for a broader investigation of brain morphology in other families with asymptomatic mutation carriers. Whether, and how, involvement of the posterior regions in asymptomatic carriers relates to ALS or FTD may be an interesting subject for further investigation. Follow-up studies are currently underway to identify possible early changes related to ALS or FTD.

Conclusions: Asymptomatic *C9orf72* carriers show cortical and subcortical differences compared to non-carriers from the same family, possibly effects of the *C9orf72* repeat expansion on the brain. Notably, changes in the primary motor regions and motor-related tracts were observed exclusively in patients with ALS, indicating that such motor changes may be a disease phenomenon. Follow-up assessment of these carriers will contribute to a better understanding of the effects of the *C9orf72* repeat expansion on the brain, an essential step for the development of preventive or therapeutic strategies.

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C70 STRUCTURAL AND FUNCTIONAL MRI SIGNATURES OF ALS PATIENTS WITH C9ORF72 HEXANUCLEOTIDE REPEAT EXPANSION

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Keywords: C9orf72, MRI, resting state functional MRI

Background: Previous studies suggest an extensive cortical and subcortical frontotemporal involvement as the *C9orf72* repeat expansion-specific structural MRI signature in ALS. However, these findings were obtained comparing C9orf72-positive patients with C9orf72-negative ALS without cognitive/behavioral impairment.

Objective: This multiparametric MRI study explored structural and functional abnormalities in C9orf72-positive ALS relative to C9orf72-negative cases with or without a comparable amount of cognitive/behavioral deficits.

Methods: Eighteen C9orf72-positive ALS patients were compared with: 22 demographically-matched healthy subjects; 22 C9orf72-negative ALS patients without cognitive impairment (C9orf72-negative ALS-motor) matched for demographics, ALSFRS-r score and disease duration; and 13 C9orf72-negative ALS patients matched for ALSFRS-r score and cognitive/behavioral deficits (C9orf72-negative ALS-plus). The presence of the GGGGCC hexanucleotide expansion in the first intron of *C9orf72* was assessed using a repeat-primed PCR assay. A cut-off of 30 repeats was used to identify a pathogenic *C9orf72* expansion. All subjects performed 3D T1-weighted, diffusion tensor (DT), and resting state (RS) fMRI. Cortical thickness measures were analyzed using surface-based morphometry. Tractography investigated white matter tracts. RS fMRI data were analyzed using a model-free approach.

Results: Relative to C9orf72-negative ALS-plus, patients with *C9orf72* hexanucleotide repeat expansion were younger and had shorter disease duration. All ALS patients showed cortical thinning of the precentral, middle/superior frontal, superior temporal, inferior parietal cortices bilaterally. C9orf72-positive and C9orf72-negative ALS-plus patients showed additional cortical thinning of inferior frontal, superior/medial parietal, and occipital regions. In the majority of cortical regions, thinning was greater in C9orf72-positive compared to C9orf72-negative ALS-motor patients, while no areas were found to be more affected in C9orf72-positive compared to C9orf72-negative ALS-plus. Compared with controls, all ALS patients showed damage to the corpus callosum, in particular the motor callosal fibers, and corticospinal tracts. Compared to C9orf72-negative ALS-motor, C9orf72-positive patients showed altered DT

MRI measures of the uncinate fasciculus bilaterally. In the sensorimotor, frontoparietal, frontostriatal and salience RS networks, C9orf72-positive patients showed increased functional connectivity relative to all other groups.

Discussion and conclusions: C9orf72-positive ALS patients showed a structural cortical and subcortical damage similar to older C9orf72-negative patients with comparable cognitive/behavioral impairment and longer disease duration, implying that frontotemporal structural damage appears earlier in C9orf72-positive than in sporadic ALS patients. In spite of similar structural abnormalities, ALS patients with *C9orf72* hexanucleotide repeat expansion showed a greater increased functional connectivity in resting state networks relative to C9orf72-negative ALS-plus cases, suggesting enhanced functional connectivity as part of C9orf72-ALS pathogenesis.

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Session 9A Motor Neuron Vulnerablility

C71 DYSREGULATION OF AXONAL RNA PROCESSING IN MOTOR NEURON DISEASE

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Keywords: axonal transcriptome, presynaptic active zone, RNA transport

Loss of motor endplates, axonal degeneration and cell death are characteristic features of motor neuron diseases. Evidence from mouse models suggests that enhanced vulnerability and sensitivity to proapoptotic stimuli is only responsible for some but not all forms of motor neuron disease (1, 2). Gene defects in FUS, TDP43, MATR-3 (3), hnRNPA1 and hnRNPA2B1 (4) in fALS and SMN in Spinal muscular atrophy (SMA) point to disturbed RNA processing in the pathophysiology of these disorders, but it is still unclear how these alterations cause synaptic loss and axonal degeneration.

The survival motor neuron (SMN) protein interacts with hnRNP R, TDP-43 and FUS and alters the RNA binding capacities of these proteins. A prominent phenotype of SMN deficient isolated motor neurons is reduced axon elongation in the absence of altered motor neuron survival (2). The axonal transport of mRNAs for beta-actin is severely reduced in SMN deficient motor neurons. In addition, the axonal transcriptome of such motor neurons lacks many transcripts for axon growth and synaptic activity (5). Also the control of local protein synthesis is dysregulated (6). The consequences are disturbed axon elongation, reduced growth cone size and functional deficits in neurotransmission that are caused by disturbed integration and clustering of voltage-gated calcium channels and other proteins for presynaptic function in axon terminals. The deficit in clustering of voltage-gated calcium channels in growth cones of SMN-deficient motor neurons and in motor neurons expressing mutant TDP-43 is accompanied by a significantly reduced frequency of spontaneous Ca^{2+} transients. Thus, common pathomechanisms in SMA and ALS may exist, and new strategies can be developed to restore altered RNA metabolism in these diseases.

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C72 PRESERVING NEUROMUSCULAR SYNAPSES IN ALS

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Keywords: agonist antibody, MuSK, neuromuscular synapses

ALS is a devastating disease, progressing from detachment of motor nerve terminals to paralytic, lethal respiratory failure within several years of diagnosis. The mechanisms responsible for axon withdrawal are poorly understood, but the loss of neuromuscular synapses is sufficient to cause muscle paralysis and therefore central to the disease. Although the subsequent loss of motor neurons has received more attention, preventing or delaying motor neuron cell death without preserving neuromuscular synapses cannot stop disease progression.

Skeletal muscles provide retrograde signals that promote the differentiation and stabilization of motor nerve terminals (1, 2). The production of retrograde signals depends upon a synaptic receptor tyrosine kinase, termed MuSK, and Lrp4, a receptor for Agrin that forms a complex with MuSK (3). Because a failure to maintain neuromuscular synapses is central to all forms of ALS, we tested whether increasing retrograde signalling in $SOD1^{G93A}$ transgenic mice would stabilize neuromuscular synapses, delay axon withdrawal and ameliorate disease symptoms (4).

We found that a modest increase in MuSK expression is sufficient to maintain neuromuscular synapses in $SOD1^{G93A}$ mice, delaying muscle denervation and improving muscle function for over one month (4). Thus, the loss of motor nerve terminals can be delayed by co-opting a retrograde signalling pathway that normally functions to stimulate the differentiation of these terminals (4). These findings suggest a novel therapeutic approach to slow the steady decline in motor function in ALS. Moreover, because motor axon withdrawal is an early, characteristic and critical feature of disease in all forms of ALS, increasing MuSK activity might provide benefit in both familial and sporadic forms of ALS.

We sought a more practical therapeutic approach to activate MuSK *in vivo*. A previous study reported that two human single chain variable region antibodies (ScFv) to MuSK, as well as IgG molecules reconstituted from these ScFv antibodies, stimulate MuSK in cultured myotubes (5). Thus, these antibodies provide an attractive means to activate MuSK *in vivo*. We found that a single injection of a humanized agonist antibody to MuSK substantially reduced denervation and increased innervation for one month. Thus, increasing MuSK activity, after denervation and disease symptoms were evident, slows synaptic loss. We are currently studying whether the agonist antibody improves motor function and whether chronic dosing with a murinized agonist antibody preserves synapses, improves

motor performance, reduces motor neuron cell death and prolongs longevity of $SOD1^{G93A}$ mice.

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C73 CONNECTING GENES TO PATHWAYS AND NETWORKS IN AN EFFORT TO REVEAL THE BASIS OF SELECTIVE MOTOR NEURON VULNERABILITY

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Keywords: upper motor neurons, systems biology, selective vulnerability

Background: To date numerous genetic mutations are identified to "cause" ALS, spinal muscular atrophy (SMA), hereditary spastic paraplegia (HSP) or primary lateral sclerosis (PLS), diseases in which different motor neurons show primary vulnerability. We hypothesize that if a mutation in a given gene leads to the development of motor neuron disease, then its protein product must be critically important for the function and health of the motor neurons that are primarily affected. Therefore connecting genes to proteins, protein-protein interactions and networks may begin to reveal the cellular events that are important for vulnerable neurons (1).

Objectives: The goal of this study is to investigate the link between genes, proteins, and networks in an effort to understand the cellular and molecular basis of selective vulnerability in motor neuron diseases.

Methods: In order to identify the networks and cellular pathways that are affected we focus on the binding partners of the proteins that are encoded by the mutated genes. We first generated an extensive data set for binding partners of proteins, whose mutated versions were reported to "cause" ALS, HSP, PLS and SMA. Similar to large-scale network analysis (2), we performed systems analysis to reveal how these proteins interact, which canonical pathways they are mainly involved in, and which cellular networks are critically important. We then identified common and unique pathways and networks in motor neuron diseases.

Results: Our preliminary studies reveal that underlying cellular events for upper and lower motor neuron vulnerability are distinct, and there are novel interaction domains for upper and lower motor neurons. The canonical pathways and key cellular events that are active in lower versus upper motor neurons show striking differences and this may in part explain why some mutations contribute to upper motor neuron vulnerability more than other mutations.

Discussion and conclusions: Data obtained from human mutations, coupled with protein network analysis shed light onto common and unique mechanisms that are responsible for upper and lower motor neuron vulnerability and degeneration. Our findings have the potential to reveal key signalling pathways responsible for selective neuronal vulnerability, and it also defines new targets for drug discovery efforts.

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Session 9B Clinical Trials

C74 CONFIRMATORY DOUBLE-BLIND, PARALLEL-GROUP, PLACEBO-CONTROLLED STUDY OF EFFICACY AND SAFETY OF EDARAVONE (MCI-186) IN ALS PATIENTS

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Keywords: edaravone, clinical trial, ALSFRS-R

Background: Edaravone is a free radical scavenger approved for treatment of acute cerebral infarction in Japan in 2001. A phase II clinical trial was conducted, and found that progression of motor dysfunction was slowed and no clinically significant adverse drug reactions occurred.

Objectives: We designed a clinical trial to confirm the efficacy and safety of edaravone in ALS patients.

Methods: We conducted a 36-week confirmatory study, consisting of 12-week pre-observation period followed by 24-week treatment period. Patients received placebo or edaravone i.v. infusion for the first 14 days in cycle 1, and for 10 of the first 14 days during cycles 2 to 6. The efficacy primary endopoint was change in the revised ALS functional rating scale (ALSFRS-R) scores during the 24-week treatment.

Results: Patients were treated with placebo (n=104) and edaravone (n=102). Changes in ALSFRS-R during the 24-week treatment were -6.35 ± 0.84 in the placebo group and -5.70 ± 0.85 in the edaravone group, with a difference of 0.65 ± 0.78 (p=0.411).

Conclusions: The reduction of ALSFRS-R was smaller in the edaravone group than in the placebo groups, but efficacy of edaravone was not demonstrated. We consider that the results are helpful to identify the patient population in which edaravone could be expected to show efficacy. On the basis of this information, we have designed and conducted a phase III study (ClinicalTrials.gov identifier: NCT01492686).

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C75 ADAPTIVE DESIGN SINGLE CENTER PHOSPHODIESTERASE TYPE 4 (PDE4) INHIBITOR –IBUDILAST (MN-166) PHASE 1B/2A CLINICAL TRIAL (NCT02238626) FOR AMYOTROPHIC LATERAL SCLEROSIS (ALS) PATIENTS (1) NOT REQUIRING NON-INVASIVE VENTILATION (NO-NIV) UP TO 5 YEARS AND (2) REQUIRING NON-INVASIVE VENTILATION (NIV) UP TO 10 YEARS FROM DISEASE ONSET

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Keywords: ALS respiratory phenotypes, non-invasive ventilation enhancement

Background: Matrix metalloproteinase-9 (MMP-9) is expressed only by fast motor neurons, which are selectively vulnerable in ALS. MMP-9 enhances ER-stress and is sufficient to trigger axonal die-back, an early pathological process in ALS models (1). Ibudilast, a phosphodiesterase type 4 (PDE4) inhibitor, can down-regulate MMP-9 (2) and suppress macrophage-migration warranting repurposing as a potential treatment for ALS patients early in ALS to enhance riluzole treatment. Ibudilast can reduce Tat-mediated transcription of tumor necrosis factor alpha (TNFα) via modulation of nuclear factor-

kappa B (NF-κB) signalling (3) and suppress the production of nitric oxide (NO), reactive oxygen species, interleukin (IL)-1beta, IL-6, and TNFα as well as enhance the production of the inhibitory cytokine, IL-10, and additional neurotrophic factors in activated microglia (4) warranting use of Ibudilast late in ALS where toll-like receptor 4 interdiction may extend survival in ALS models (5). 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 ALS stages (early-distal axonopathy; late-microglial activation) (6, 7).

Objective: Report on implementation and results of adaptive design for single center Ibudilast phase 1b/2a clinical trial in ALS patients: 1) not requiring non-invasive ventilation (no-NIV) up-to-5 years of disease; and 2) requiring non-invasive ventilation (NIV) up-to-10 years from disease onset.

Methods: This study was a Single-Center (2:1), Randomized Delayed-Start, Double-Blind, Placebo-Controlled, Six-Month Clinical Trial followed by an Open-Label Extension to evaluate the feasibility, tolerability, safety and clinical endpoint responsiveness (strength; ALSFRS-; slow vital capacity) of Ibudilast, assessed during standard of care encounters at 3-month intervals with intervening telephony adverse event/concomitant medication reviews in 60 no-NIV and 60 NIV subjects, employing novel statistical techniques.

Results: Achievement of 1st milestone - safety report at 3 months Ibudilast exposure in no-NIV subjects (1b goal-AAN, 2015) (31 patients: Treated stage 1 hypertension present in both MN-166 treated and placebo subjects with no differential cluster of adverse events/concomitant medications in either group; No clinically significant electrocardiogram or clinical laboratory abnormalities; Feasibility – 29/31=93/5%; Tolerability – 28/31 = 90.3%; Safety-1-Serious Adverse Event Rate (1/31) = 96.8%; Reduced rate of loss of vital capacity-%-predicted loss per month (-1.3 + 7.7 (SD)) compared with Pro-ACT database (-2.2 + 6.9)); 2nd milestone - safety report at 6 months Ibudilast exposure (31 no-NIV subjects) (1b goal-ALS - MND, 2015); 3rd milestone - clinical endpoint responsiveness at 6 months (31 no-NIV subjects) (2a goal-ALS-MND, 2015); 4th milestone - safety report at 3 months Ibudilast exposure in NIV subjects (30 patients) (1b goal-ALS MND, 2015).

Conclusions: Ibudilast administration is feasible, tolerable and safe when administered over 3 months to no-NIV ALS subjects. Longer feasibility, tolerability and safety data will be reported in no-NIV ALS subjects over 6 months. Feasibility, tolerability and safety of Ibudilast treatment in advanced NIV ALS subjects will provide, for the first time, information regarding the participation of advanced ALS patients in clinical trials of pharmacological agents at this stage of ALS.

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C76 ULTRA-HIGH DOSE METHYLCOBALAMIN (E0302) PROLONGS SURVIVAL OF ALS BY MORE IF TREATED EARLY: RANDOMIZED DOUBLE-BLIND, PHASE 3 CLINICAL TRIAL (CLINICALTRIALS.GOV NCT00444613)

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Keywords: methylcobalaminw; RCT, survival

Objective: To investigate the efficacy and safety of ultra-high dose (25mg or 50mg i.m. twice weekly) of methylcobalamin compared with placebo for amyotrophic lateral sclerosis (ALS) patients.

Background: High-dose methylcobalamin showed neuroprotective effects in acrylamide neuropathy (1) and the increase in compound muscle action potential in a trial for ALS (2).

Methods: Patients (373) who were diagnosed with definite, probable, or probable-laboratory-supported ALS by revised El Escorial criteria were enrolled in this study. Those with FVC less than 60% and the disease duration more than 3 years were excluded. Patients were randomly assigned to receive placebo, 25mg, or 50mg methylcobalamin i.m. twice weekly for 182 weeks. Primary endpoints were event-free survival (time until death, TIPPV or all-day NIPPV) and ALS Functional Rating Scale-Revised (ALSFRS-R) changes.

Results: Of 373 patients, 370 constituted the full analysis set (placebo n =123; 50mg n=123; 25mg n=124). In both endpoints, there was no statistical significance in the comparison for the two dose response contrasts (linear and saturate hypothesis). For the patients who were given a diagnosis of ALS within 12 months after the onset (placebo n=48; 25mg n=54; 50mg n=42), the event-free survival was prolonged in a dose-dependent manner (P=0.010, hazard ratio (95% CI) vs 25mg, 50mg were 0.640 (0.377, 1.085), 0.498 (0.267, 0.929), respectively) and ALSFRS-R changes were smaller in active groups (P=0.003) than in placebo. No adverse events of particular concern were noted.

Discussion: The diagnosis of ALS with revised El Escorial criteria is often delayed but newly-devised Awaji criteria may enable earlier diagnosis. Patients are less likely to benefit from ultra-high dose methylcobalamin treatment if more than 2 to 3 years have passed since the onset of ALS.

Conclusion: The present study indicates that ultra-high dose methylcobalamin can significantly prolong survival and retard progression in ALS if administered early.

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C77 A PHASE 2 STUDY FOR SAFETY AND EFFICACY EVALUATION OF TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS USING AUTOLOGOUS BONE-MARROW-DERIVED STROMAL CELL

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Keywords: bone marrow-derived mesenchymal stromal cells (MSCs), stem cell therapy, clinical trial

Background: There is no effective treatment and many previous efforts using various neuroprotective agents did not prove successful in ALS. Recently, stem cell-based therapy is an emerging alternative therapeutic or disease-modifying strategy in ALS.

Objectives: On the basis of our previous preclinical and clinical trial, we sought to evaluate the safety and feasibility of repeated intrathecal administrations of autologous bone marrow-derived MSCs in patients with ALS.

Methods: This study was designed as a single center, randomized, parallel-group, phase 2 trial (HYUH IRB 2010-C-70, KFDA-2413, clinicaltrials.gov: ID NCT01363401). 64 patients (treatment group: 33, control group: 31) were enrolled. 59 patients (treatment group: 32, control group: 27) were analysed. After a lead-in period for 3 months, autologous MSCs were isolated from bone marrow, expanded *in vitro* and suspended in autologous CSF, 32 patients received an intrathecal MSCs (1×10^6/kg) injection twice at an interval of 1 month via standard lumbar puncture. After the first MSC injection, clinical and laboratory measurements were recorded to evaluate its safety. Primary outcome measures the decline rate of ALSFRS-R score from baseline to 4 months. Occurrences of AE and SAE, all clinical and laboratory findings were collected for safety analysis. The changes of Appel score, forced vital capacity (FVC) were the secondary outcome.

Results: No significant major adverse events (AE) were reported. MSCs injection was well tolerated except for occurrences of transient headache, myalgia, and back pain. These AEs disappeared spontaneously or with simple analgesics within 1 or 2 weeks. ALSFRS-R decline rate was significant lower in treatment group compared to control group during the first 4 months and 6 months follow-up period (0.42 ± 0.64/month vs. 1.17 ± 0.81/month, p=0.0002, 0.58 ± 0.68/month vs. 1.25 ± 0.90/month, p=0.003). Appel score change 4 months after MSCs injection compared to baseline was showing statistically significant differences between treatment group and control group (10.44 ± 9.24 vs. 17.96 ± 11.78, $p < 0.0091$).

Discussion and conclusions: Repeated intrathecal injections of autologous bone marrow-derived MSCs was safe and feasible in patients with ALS. The changes of ALSFRS-R scores suggest a trend towards stabilization. However, the benefit needs to be confirmed in the following large-scale study and long-term follow-up period.

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Session 10A Disease Mechanisms and Therapeutic Targets

C78 DIRECT CONVERSION AS A METHOD TO SUBGROUP ALS PATIENT POPULATIONS

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Keywords: reprogramming, C9orf72 *repeat expansion*, *non-cell autonomous effects*

One of the major difficulties in designing clinical trials or understanding disease mechanisms in ALS and other neurodegenerative disorders is the heterogeneity of the patient population. Currently, biomarkers to distinguish fast progressing from slow progressing patients as well as to predict their potential reactivity to new therapeutics are lacking. Methods that allow subgrouping patients will likely help to improve our understanding of the disease and accelerate the pathway towards finding new therapeutic targets.

We have recently published a fast and efficient reprogramming method that allows direct conversion of skin fibroblasts into induced neuronal progenitor cells (iNPCs) as a new model system for ALS (1). We have extensively used this system to expand the lines of iNPCs and to produce astrocytes, oligodendrocytes and motor neurons from them. We find that both, astrocytes and oligodendrocytes from ALS patients reduce survival of co-cultured mouse and human motor neurons and induce axonal beading and neurite retraction with varying aggressiveness. Strikingly, the extent of motor neuron death in co-culture showed a strong correlation to the disease duration of the donating patients ($p<0.001$). Moreover, for most sporadic ALS cases as well as SOD1 mutation carriers, we identified aggregates of misfolded SOD1 protein by immunofluorescence using several different antibodies, as well as immunopulldowns.

Consequently, the motor neuron survival was rescued by reducing SOD1 levels in astrocytes or oligodendrocytes derived from these patients. In contrast, *C9orf72* repeat expansion carrying astrocytes or oligodendrocytes did not contain misfolded SOD1 and the severity by which these cells affected the survival of motor neurons was not ameliorated by reduction of SOD1. Instead, they displayed both nuclear and cytoplasmic RNA foci. Using the new genome-editing tool CRISPR-CAS9, we were able to excise the repeat expansion in iNPCs. Interestingly, astrocytes and oligodendrocytes derived from these treated cell lines showed reduced accumulation of RNA foci in their nuclei as well as reduced toxicity towards motor neurons.

Our data strongly support that this *in vitro* system is suited to study disease mechanisms involved in ALS and suggests that ALS patients can be subgrouped based on either disease severity or reaction to certain treatments.

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C79 A TRANSCRIPTIONAL COMPARISON OF HUMAN IPSC AND MOUSE MODELS OF ALS DEFINES THE IMPACT OF MOTOR NEURON MATURATION, AGING AND DISEASE

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Keywords: iPSCs, *transcriptomics*, *maturation*

Modeling neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) with human induced pluripotent stem cells (iPSCs) or with transgenic mice aims to re-enact the embryonic development, maturation, aging, and subsequent degeneration of spinal motor neurons (spMNs). Several studies have reported molecular pathways affected by ALS conditions in both model systems as well as in post-mortem human spMNs. However, the extent to which these ALS models faithfully capture pathways observed in human spMNs has not been closely examined. Specifically, the maturity of human spMNs derived from iPSCs *in vitro* has not been directly compared to human spMNs *in vivo*, and whether a difference in maturity levels impacts faithful disease modeling has yet to be addressed. Furthermore, while transgenic mice have effectively modeled genetic forms of ALS, it is unclear to what extent this *in vivo* system faithfully captures all aspects of human spMN development and degeneration.

Here, we compared global transcriptional profiles among human iPSC-derived spMNs, fetal and adult spinal tissues as well as orthologous tissues from mice. By comparing gene expression changes in ALS conditions across iPSC-derived spMNs, human spinal cord tissue and mouse spMNs, we highlight that iPSC and mouse ALS

models affect some shared and some distinct pathways. Our comparative analysis among cells spanning embryonic to adult spMN states indicated that iPSC-derived spMNs are similar to fetal rather than aged adult spinal tissue. Additionally, gene co-expression network analysis identified gene modules that tightly associate with spMN fetal development, maturation, or aging. Interestingly, some of these modules enrich for clinical spMN disease genetic variants, revealing that maturation and age-related pathways may play roles in disease presentation. Collectively, these analyses suggest that more effective iPSC models of ALS necessitate strategies to further mature and age iPSC-derived spMNs. Lastly, we demonstrate that while the global expression of orthologous genes involved in spMN fetal development and maturation are largely parallel between human and mouse systems, only maturation but not fetal development gene co-expression networks are well-conserved between the two species. This analysis thus provides a more sensitive interrogation of gene-to-gene expression relationships underlying human and mouse spMN physiology. Overall, our findings support the idea that iPSC and mouse models of ALS are useful systems to study motor neuron development and degeneration, but also highlight important differences that can guide more effective disease modelling.

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C80 USING C. ELEGANS TO IDENTIFY CONSERVED MODIFIERS OF C9ORF72-ASSOCIATED DIPEPTIDE TOXICITY

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Keywords: C. elegans, C9orf72, dipeptide

Nucleotide repeat expansions are a common cause of age-related neurodegenerative diseases. An expansion of the intronic hexanucleotide repeat GGGGCC in the *C9orf72* gene is associated with two major neurodegenerative diseases - amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). Both repeat containing RNA and repeat encoded dipeptide proteins are associated with neurotoxicity and death in *Drosophila* and mammalian cell culture (1-3). To gain insights into the mechanisms of dipeptide toxicity, we generated transgenic *C. elegans* expressing dipeptides fused to GFP. We focused solely on the effects of dipeptide toxicity by varying codon usage, which preserves the dipeptide coding sequence but eliminates potential RNA repeat induced toxicity. Dipeptide expression was controlled by promoters that are active in muscle, all neurons, or specific motor neurons. Expression of GR and PR, but not GA and PA, was extremely toxic under all conditions, producing various phenotypes (lethality, short lifespan, motility defects) depending on the site of expression. Since there are no known modifiers of dipeptide toxicity, we used these phenotypes to perform biased and unbiased modifier screens. One modifier we identified was a mutation in the *daf-2* gene, which encodes the sole *C. elegans* insulin/IGF receptor. Mutations in *daf-2* activate the insulin signalling pathway, which is an established modifier of longevity and proteotoxicity from *C. elegans* to humans and is known to reduce toxicity in other *C. elegans* ALS models (4). Therefore, dipeptide toxicity and other forms of ALS may share a common mechanism(s) of toxicity that is opposed by the target(s) of insulin signalling. We also performed unbiased genetic screens for modifiers of dipeptide toxicity. A small pilot screen identified one mutant that suppressed GR dipeptide toxicity. This mutant does not appear to act via insulin/IGF signalling, suggesting that it suppresses dipeptide toxicity through a unique mechanism(s). Detailed characterization of these dipeptide suppressor mutants is ongoing.

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C81 *IN VIVO* CHEMIOGENETICS REVEALS NEUROPROTECTIVE EXCITATION-RELATED SIGNALING IN NEURONS AND ASTROCYTES IN SOD1 (G93A) MOUSE

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Keywords: excitation, dreadds, astrocytes

Background: The full-blown phenotype of the SOD1^{G93A} ALS model is known to be non-cell-autonomous and requires the interplay of neuronal and non-neuronal components. The precise role of each cell type is still incompletely understood because tools to manipulate neurons and glia independently with high specificity were so far unavailable. Recently, direct control of neuronal activity has revealed that excitation and activity pattern of motor neurons is a key player in determining disease vulnerability and in delivering neuroprotection (1, 2). Thus, neuronal activity may be a site of integration of multiple converging pathogenic events in ALS since it is influenced by synaptic and non-synaptic plasticity in neurons, by activity-regulated signalling cascades, and by activity-related response in astrocytes. Independent control of each of these components can now be achieved by chemiogenetics with orthogonal pharmacology, in particular by the combinatorial application of engineered ion

channels and designer GPCR (DREADDs) *in vivo* with high cellular specificity and spatio-temporal resolution (3).

Objectives: To explore of excitation-related processes in the spinal cord of the $SOD1^{G93A}$ ALS model by using DREADDS to control PKA, PKC and Ca^{2+} in motor neurons and in astrocytes *in vivo*.

Methods: Evolved GPCR (DREADDs) are expressed in astrocytes *in vivo* by injection of AAV8-expressing DREADD under the control of the GFAP promoter. Different DREADDS are espressed in motoneurons by injecting AAV8 or AAV9-expressing DIO-DREADDs in $SOD1^{G93A}$; ChAT-CRE double-transgenic mice. Multiple AAVs are co-injected in multiplexer chemiogenetic experiments. Intraspinal surgery is performed at P19-20, agonist administration starts at P25 and lasts for five days. Agonist (CNO, 1mg/kg; vehicle as control) is administered intraperitoneally twice daily.

Results: Activation of Gs-PKA-coupled DREADD in motor neurons is sufficient in rescuing a number of biochemical abnormalities: decreased misfolded SOD1 burden; reduced ER stress; normalized mitochondrial morphology; and clearing of p62 aggregates are observed after 5 days of treatment. In DREADD-positive motor neurons, PKA activation results in increased density of inhibitory terminals and normalized size of cholinergic boutons. The activation of Gq-coupled DREADD (but not of Gs- or Gi-coupled DREADDs) in astrocytes, results in the decrease of misfolded SOD1; autophagy impairment; and mitochondrial abnormalities. Both effects are abolished by simultaneous decrease in motor neuron firing in double-chemiogenetic experiments.

Discussion and conclusions: Cell-specific manipulation of signalling cascades via DREADDs reveals that PKA-mediated cascade in motor neurons is critical in delivering activity-related neuroprotective effects. Likewise, activity-dependent neuroprotection is achieved by increasing PKC/Ca^{2+} in spinal cord astrocytes.

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C82 BROMODOMAIN INHIBITORS REGULATE THE C9ORF72 LOCUS IN ALS

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Keywords: C9orf72, epigenetic, therapeutics

A hexanucleotide repeat expansion residing within the *C9ORF72* gene represents the most common known cause of ALS and places the disease among a growing family of repeat expansion disorders. Evidence supports multiple contributors of *C9ORF72*-related pathology which include the production of toxic RNAs, Repeat Associated Non-ATG Translation (RANT) products and haploinsufficiency due to the reduced *C9ORF72* expression via an epigenetic mechanism. It has been proposed that such epigenetic alterations contribute to the pathogenesis of several repeat-expansion disorders described to date, including *C9ORF72*-ALS, Fragile X syndrome, and Friedreich's ataxia. The evidence that epigenetic perturbations play a role in the pathophysiology of *C9ORF72*-ALS derives, in part, from its analogy to these other intronic repeat expansion disorders and from the observation that expression of the *C9ORF72* gene is reduced. Furthermore, recent empirical evidence has shown that expanded *C9ORF72* alleles are associated with repressive epigenetic markers including histone-3-lysine-9-trimethylation (H3K9me3) and DNA hypermethylation of the promoter. Taken together, these observations indicate that the *C9ORF72* expansion event alters the local epigenetic environment such that the rate of transcription from the expanded allele is reduced in patient cells and tissues.

Small molecule histone deacetylase (HDAC) inhibitors have been shown to significantly reduce disease phenotypes in Friedreich's ataxia animal models and have been tested in patients. This sets a precedent for small molecule epigenetic compounds being potential tools in the treatment of repeat expansion disorders, although translation from therapeutic proof-of-concept to clinical trials has been modest. There have now been remarkable advancements in developing small molecules that target classes of epigenetic proteins other than HDACs. One example is the bromodomain-extra terminal (BET) family of bromodomain proteins. While they lack catalytic activity, BETs bind to acetylated histones and function as epigenetic "reader" proteins. Novel small molecule BET inhibitors effectively displace BET proteins from acetylated histones and elements of the transcriptional machinery; they have shown efficacy in mouse models of inflammation, cancer, viral infection and a variety of other indications. Examples include JQ1, I-BET-762 and I-BET151 which exhibit specificity for the BET proteins, particularly BRD2, BRD3 and BRD4 over other bromodomain-containing proteins and epigenetic enzymes.

We utilized a semi-high-throughput gene expression based screen to identify siRNAs and small molecule inhibitors of epigenetic modifier proteins that regulate *C9ORF72* RNA in patient fibroblasts, lymphocytes and reprogrammed motor neurons. We found that several bromodomain small molecule inhibitors increase the expression of *C9ORF72* mRNA and pre-mRNA without affecting repressive epigenetic signatures of expanded *C9ORF72* alleles. These data suggest that bromodomain inhibition increases the expression of unexpanded *C9ORF72* alleles in patient cells and may therefore hold therapeutic value for this disease.

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C83 GENETIC REMOVAL OF HISTONE DEACETYLASE 6 (HDAC6) DELAYS THE DISEASE PROGRESSION IN A FUS MOUSE MODEL OF ALS

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Keywords: FUS/TLS, axonal transport, transgenic mouse model

Background: Fused in sarcoma (FUS) is an RNA-binding protein implicated in the pathogenesis of ALS. FUS aggregates are observed in neuronal and glial cells of ALS patients and mutations in FUS are identified in 4% of familial ALS and in 1% of sporadic ALS cases. Recently, a mouse model based on FUS resembling an ALS phenotype was published. Mice overexpressing human wild type (wt) FUS under the control of the prion promoter (PrP-hFUS-WT) developed an aggressive ALS-like phenotype with symptoms occurring at four weeks of age and end-stage around the age of eight weeks. Previously, we reported a beneficial effect of genetic removal of histone deacetylase 6 (Hdac6) in the mutant $SOD1^{G93A}$ mouse model of ALS. In contrast to the other Hdacs, Hdac6 is a cytoplasmic enzyme responsible for the deacetylation of cytoplasmic substrates. One of the most important substrates of Hdac6 is α-tubulin and deacetylation of α-tubulin has a negative effect on axonal transport.

Objectives: The aim of this study was to find out whether genetic removal of Hdac6 has an effect on the disease phenotype of the new PrP-hFUS-WT mouse model.

Methods: PrP-hFUS-WT mice were crossbred with Hdac6 KO mice to create double transgenic mouse strain. Disease progression was monitored from three weeks of age on by weight, rotarod, hanging wire, muscle strength, electromyography and survival. Neural tissues were collected from end-stage mice and analyzed with Western blot for FUS expression and α-tubulin acetylation.

Results: Heterozygous Hdac6 knock-out prolonged significantly the average survival of PrP-hFUS-WT mice with 14 days, from 57.2 ± 2.3 to 70.9 ± 4.3 days. Symptom onset age was not delayed in heterozygous Hdac6 knock-out mice when assessed by hanging wire, while motor coordination was slightly improved. In addition, partial Hdac6 deletion had a positive effect on the amplitude of the compound muscle action potential (CMAP). Hdac6 deletion did not affect the expression levels of human or endogenous FUS. In contrast, heterozygous Hdac6 knock-out significantly increased the acetylation of α-tubulin in the sciatic nerve of transgenic FUS mice.

Discussion and conclusions: Transgenic mice overexpressing wt hFUS have a severe ALS-like phenotype that could be ameliorated by genetic removal of Hdac6. The effect on the survival by Hdac6 deletion was 24%. The beneficial effect of Hdac6 deletion was not due to changes in the FUS expression levels but instead increase in α-tubulin acetylation. We conclude that the positive effect of genetic deletion of Hdac6 is present in at least two different mouse models of ALS. As a consequence, Hdac6 could be an interesting therapeutic target.

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C84 PROTECTION BY COPPER DELIVERY IN SOD-TRANSGENIC MICE AND THE IMPORTANCE OF THE COPPER CHAPERONE FOR SOD1 (CCS)

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Keywords: superoxide dismutase, copper, CCS

Background: Paradoxically, co-expression of the human copper chaperone for SOD1 (hCCS) accelerates death in hi-expressing SOD^{G93A} mice. Last year we reported that CuATSM was extremely protective in these mice. Most of the treated mice continue to survive with minor symptoms.

Objectives: Determine how CuATSM protects CCS x SOD^{G93A} mice from developing ALS and understand what happens to reinitiate the disease when the drug is withdrawn.

Methods: Randomized and blinded trials of CCS x SOD^{G93A} mice were conducted with CuATSM withdrawn to initiate disease and restarted after mice developed symptoms. SOD was measured in spinal cords of mice by mass spectrometry and cytochrome *c* oxidase by spectrometric assays.

Results: CuATSM given to high-expressing Gurney SOD^{G93A} mice coexpressing the human Copper Chaperone for SOD (CCS) protected against ALS for 20 months with many mice still surviving or being censured for unrelated issues. When CuATSM treatment was stopped, the CCS x SOD^{G93A} mice began showing severe motor dysfunction in two months and died in the next month. Progression could be stopped by resuming treatment with CuATSM. These rescued mice largely recovered and survived for an additional 6-12 months. Mitochondrial cytochrome *c* oxidase was decreased to 25% in untreated CCS x SOD^{G93A} mice and in mice where CuATSM was withdrawn. Activity was restored by long-term CuATSM treatment to levels found in non-transgenic mice. Mass spectrometry was used to measure native SOD directly in ventral spinal cord, and showed

that CuATSM treatment completed maturation to the fully functional Cu, Zn SOD in CCS x SOD^{G93A} mice and that SOD protein doubled in concentration compared to end-stage SOD^{G93A} mice.

Conclusions: CuATSM protects CCS x SOD^{G93A} mice by at least two mechanisms; supplying copper to cytochrome *c* oxidase and completing the maturation of SOD to its mature form containing copper and zinc. All humans ALS patients likely express CCS, making this model closer to the human condition than the standard ALS model. CuATSM is remarkably nontoxic and is in use in humans now.

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Session 10B Cognitive Change

C85 AMYOTROPHIC LATERAL SCLEROSIS AND FRONTOTEMPORAL DEMENTIA: UNDERSTANDING THE SPECTRUM

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Keywords: frontotemporal dysfunction, neural networks, neuropsychology

While ALS is classically defined as a progressive degenerative disorder affecting primarily the motor neurons, it is increasingly clear that not only is ALS syndromic at both the cellular and clinical level but that there is considerable overlap for frontotemporal dysfunction. As such, approximately half of all patients will develop a more broadly based degenerative process in which both cortical and subcortical degeneration gives rise to either a frontotemporal dementia, a syndrome of behavioural or cognitive impairment, or impairments in emotional intelligence/ Theory of Mind. While the exact processes giving rise to these deficits continue to be defined, recent evidence suggests that a fundamental disruption of neural networks may underlie the clinical deficits. Less certain is the mechanism(s) by which this disruption can occur and what role, if any, genetics has in this process. However, given the significant impact of frontotemporal dysfunction on the disease course in ALS, future clinical trials will need to be stratified based on the presence or absence of frontotemporal dysfunction on the disease course of ALS. In this lecture, we will review the clinical, neuropathological and molecular understandings of frontotemporal dysfunction in ALS.

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C86 A PROSPECTIVE POPULATION-BASED INVESTIGATION OF CROSS-MODAL EMOTIONAL PROCESSING IN ALS

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Keywords: cognition, emotion, social cognition

Background: Executive Dysfunction is known to occur in early stages of Amyotrophic Lateral Sclerosis (ALS), and it is widely accepted that ALS and Frontotemporal Dementia (FTD) lie on a cognitive-behavioural continuum. Social cognitive processes are considered by some to be subsumed by executive functions, although deficits in social cognition have been reported in a select cohort of ALS patients without executive dysfunction. Emotional identification and recognition using visual stimuli in ALS, has recently been shown to be an effective marker in discriminating between ALS and ALS-FTD, but little is known about the extent of these deficits in multi-modality testing.

Objective: To investigate ALS neuropsychological profiles relative to executive function and social cognition, with supported multi-modal (verbal, visual and cross-modal) assessments of emotional processing.

Methods: The present study investigated affective social cognitive deficits in patients with ALS, accompanied by detailed measures of executive function, language, as well as auditory, visual and cross-modal assessments of affect and emotion (N=70). Participants were recruited as part of a population based study investigating heterogeneous cognitive profiles in ALS, within Ireland. After removing patients who were positive for the pathogenic *C9orf72* hexanucleotide repeat, participant data were analysed on a group level and further sub-stratified based on bulbar or spinal onset ALS. Gender-, age-, IQ- and education-matched healthy controls were recruited to generate culturally specific comparative data.

Results: On a measure of social cognition commonly used in ALS, there was a significant difference between bulbar and spinal onset patients on this task ($p < 0.001$), although not at a group level when compared to controls. Preliminary data comparing bulbar and spinal patients standardized scores of executive function yielded no significant differences. Further analyses of the emotion-specific data suggest that patients with ALS perform worse than controls on executively loaded subtests, and that the modality of testing did not have an effect (auditory visual stimuli). Patients performed significantly lower when required to identify an emotion on an incongruous task ie, a negative sentence said in a positive emotional prosody.

Discussion and conclusion: These scores may illustrate a decline in social cognition may predate higher order functional deficits in ALS. Notwithstanding, these results will be discussed within the context of social affect measurement tools, and their psychometric properties.

We conclude with the argument that although ALS patients are known to have social cognitive deficits, semantically based test-taking strategies may confound results within this cohort, and deficits may be more prevalent than testing would suggest.

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C87 THE SOCIAL BRAIN OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS): THE MORE THE BETTER

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Keywords: social support, emotion, quality of life (QoL)

Background: Amyotrophic lateral sclerosis (ALS) is known to affect emotional processing. Negative cues in socio-emotional pictures are less recognized and ability to empathize other's intention in theory of mind tasks is reduced.

Objectives: Emotional processing of facial cues was measured in ALS patients compared to healthy subjects by using fMRI and associated to depressiveness and number of social contacts of patients.

Methods: Processing of emotions in faces was investigated in 30 ALS patients and 29 age, education and gender matched healthy subjects with a behavioural test of Ekman faces. A subset of 15 ALS patients and 14 matched healthy controls performed a similar task during fMRI. Sixty Ekman faces were presented with emotional expressions of disgust, fear, sadness, surprise, anger and happiness and compared to "meaningless" stimuli with coloured random scattered patterns. Depression (ADI 12) and social activity (in-house questionnaires) were measured.

Results: ALS patients recognized disgust and fear less accurately than healthy subjects. Variance in sadness rating was explained by depressiveness score. During fMRI task of processing facial expressions, reduced brain activity was seen in left inferior parietal cortex, left middle temporal and left precentral gyrus. Furthermore, reduced activity was seen in left inferior frontal gyrus.

Increased brain activity was seen in areas associated with mirror neurons ie in right sided inferior frontal gyrus in ALS patients compared to healthy subjects. This increased activity was specifically associated with processing of sad faces, for which ALS patients presented also a lower activity in hippocampus bilaterally. No increased brain activity was seen for any of the other emotional expressions. Additionally, the possibly compensatory mirror neuron activity was associated with increased amount of social contacts.

Discussion and conclusions: ALS patients presented decreased activity in cortical areas involved in processing of disgust and fear; ALS patients had more difficulties in recognizing facial emotions. This might be interpreted as a general deficit in the network of facial emotion processing. Furthermore, for sad facial emotions for which they had no difficulties recognizing, ALS patients presented with increased activity in cortical areas associated with mirror neurons and therefore key structures for facial emotion processing. The increased activity in this area might represent a compensatory process. Interestingly, activation in these areas was associated with the amount of social contacts of an ALS patient, suggesting that positive impact of social contacts on affective state (depressiveness) might counteract the deficits in cortical network of facial emotion processing in ALS.

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C88 RELATIONSHIP BETWEEN COGNITIVE AND BEHAVIORAL IMPAIRMENT AND DEPRESSION IN A LARGE ALS COHORT

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Keywords: depression, cognitive function, behavioural impairment

Background: While an association between cognitive deficits and depression repeatedly has been shown in medically healthy depressed patients, little is known about such an association for patients with ALS.

Objective: To integrate findings on cognitive-behavioral and psychiatric/psychosocial measures observed at the baseline visit of a national cohort of patients with ALS.

Methods: Patients with definite or probable ALS diagnoses, diagnosed within the past 18 months at 16 clinics across the U.S. were enrolled between 2010 and April 2013. Neuropsychological tests were conducted at each site while the balance of assessments was conducted by Columbia interviewers by telephone. Neuropsychological measures included the ALS Cognitive Behavioral Scale (CBS) and the Frontal Behavioral Inventory (FBI). Seven psychiatric-psychosocial measures were administered, including the Patient Health Questionnaire (PHQ) for diagnosis of depression. Cognitive impairment classification (absent, mild, moderate) was based on the Cognitive subscale of the CBS (covering areas including verbal or written word fluency, attention, memory), while behavioral impairment was based on the Behavioral subscale (with items such as impulsivity, apathy, irritability). Depression diagnosis using the PHQ was classified as present/absent and includes both major and minor depressive disorder because of small numbers. Wish to die was assessed by responses to the PHQ item, "thoughts you would be better off dead or of ending your life".

Results: 253 patients completed all measures. Of these, 79 patients (32%) had neither cognitive nor behavioral impairment, 100 (45%) had cognitive impairment, 23 (9%) had behavioral impairment, and 45 (18%) were

impaired on both cognitive and behavioral measures. When cognitive impairment was present, it was in the mild range for 90% and severe for 10%. Thirty-one patients (12%) had major or minor depressive disorder (DSM-IV criteria). Cognitive impairment level was unrelated to a diagnosis of depression or any other measure of distress including positive and negative mood, hopelessness, quality of life, stress, or spousal support. Scores on the FBI were also unrelated to cognitive impairment. In contrast, patients with behavioral impairment, with or without cognitive impairment, reported more depressive symptoms, lower scores on positive mood and higher scores on negative mood, had higher scores on measures of hopelessness and stress, and reported more negative support from spouse/caregiver. Consistent with this pattern, scores on the FBI were also related to behavioral impairment. Wish to die was unrelated to either cognitive or behavioral impairment, or to the FBI.

Discussion: We found no association between cognitive impairment and depression or any other measure of distress we evaluated. In contrast, behavioral impairment was strongly associated with depressive symptoms and diagnoses. Thoughts about ending life or being better off dead appear unrelated to either cognitive or behavioral changes associated with ALS and may reflect personal values rather than psychopathology.

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C89 NEUROPSYCHIATRIC SYMPTOMS PREDICTIVE OF GREATER CAREGIVER DISTRESS IN ALS

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Keywords: neuropsychiatric symptoms, non-motor changes, apathy

Background: The recognition of pervasive neuropsychiatric symptoms in ALS and their impact on caregiver burden has gained momentum in recent years. A scientific understanding of their impact is critical for the development of tailored services, future trials, and for improving quality of life.

Objective: To investigate the impact of neuropsychiatric symptoms on caregivers of patients with ALS Behavioural versus ALS Motor.

Methods: 53 consecutive ALS patients seen at the MND Multidisciplinary Care Centre in Cambridge, UK, from November 2014 to May 2015. Patients were sub-classified into ALS behavioural and ALS motor according current ALSFTD criteria, based on the Motor Neurone Disease Behavioural Scale (MiND-B, max score 36, cut off <34). The Cambridge Behavioural Inventory was applied to investigate changes in 8 domains (Memory, Disinhibition, Mood, Abnormal beliefs, Eating changes, Sleep, Stereotypical behaviour, and Apathy) as well as the impact of these changes on the family caregiver.

Results: 30 patients with ALS Motor and 23 patients with ALS Behavioural were matched for age (M=66.1, SD=12.5), gender (approximately 42% females in both groups) and disease duration (M=39.3 months; SD=39). Frequency of neuropsychiatric symptoms was significantly higher for ALS Behavioural on all CBI-R domains except for abnormal beliefs. Caregiver distress, however, was significantly higher for specific CBI-R domains: Memory; Sleep, Stereotypical behaviour and Apathy. Further within group analyses revealed that domains leading to most caregiver distress were changes in Sleep and Mood for all ALS caregivers, but Apathy was also highly distressing for caregivers of ALS Behavioural patients.

Conclusions: a systematic approach to investigating the impact of classical and non-motor symptoms in ALS can expose key areas to be targeted clinically, and in pharmacological and non-pharmacological trials. This study demonstrates the pressing need to include non-motor symptoms as outcome measures in future trials.

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Session 11 Joint Closing Session

C90 STEM CELL TREATMENT STRATEGIES FOR NEURODEGENERATIVE DISEASE: FROM HYPE TO HOPE?

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Keywords: stem cells, disease model, cell therapy

For a while stem cell therapies had become the new snake oil for ALS – promises of cures based on hype rather than hope. Today the field has developed to a stage where stem cells now play an important role in the treatment of ALS. Their utility lies in two different areas. The first is to generate induced pluripotent stem cells (iPSCs) from ALS patients and differentiate them into motor neurons and glial cells in order to model the disease in the petri dish. New drugs can be tested on these motor neurons (rather than the patient) which should lead to better success in the clinic. We are involved with a large program generating 1000 iPSC lines from ALS patients – the largest single set created to date. These will be used in large omics studies to understand more about the molecular mechanisms of disease. There is a great need to increase the physiological relevance of these models. We are developing exciting new methods utilizing "organ on a chip" technology where layers of blood vessels are aligned next to the ALS motor neurons and microfluidics simulate blood flow. We hope that these newer petri dish systems will provide a better model of the ALS for drug discovery and mechanistic insights. The second utility is of course to use stem cells as therapeutic agents. There are a number of active stem cell clinical trials in progress or completed, along with some others that are almost ready to start. These include our own CIRM funded trial at Cedars-Sinai that will use stem cells modified to release the powerful growth factor GDNF. This trial is scheduled to begin in early 2016. Together, these stem cell studies now offer real hope for ALS patients.

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C91 PATIENT 3.0, THE CENTRE OF DRUG DEVELOPMENT

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Keywords: patient 3.0, innovation, collaboration

Over five years ago, I was unexpectedly struck by amyotrophic lateral sclerosis, or ALS. I have dedicated my life to moving mountains in what was, at first, an unfamiliar field. My entrepreneurial career included real estate, flowers, oil and shipping. But the past five years has challenged me with the steepest learning curve I've ever faced: my disease.

Although I am an academic drop out, I've learned to appreciate science as the backbone of evidence-based theories. However, being a patient with a killer disease means never ruling out miracles - the desire for a cure drives everything I've come to appreciate the passion and compassion of people within the ALS community - the patients, researchers and companies that underpin that drive.

I apply the skills I developed in my previous careers to drive innovation in drug development. This has led to the development of what is currently the largest genetic dataset of ALS patients on the globe. The shift in focus from technology-driven to disease-driven drug development is necessary in an industry ruled by science but governed by non-disclosure agreements. The heart of next generation drug development lies here - with innovative research approaches that include the experiences of the patients that the industry is focused on helping.

Today, real innovation increasingly comes as a result of patients' involvement in drug development. Admittedly, most pharma and biotech companies have patient-centric advocacy embedded in their strategy and mission statements, but patients have traditionally formed just a small part of the drug development equation. The patient voice should echo in all parts of the development path, including governing bodies, ethical committees and expert panels.

Patients are increasingly aware of their ability to play a significant role in the biotech R&D paradigm. Social phenomena like the Ice Bucket Challenge and increased patient involvement to accelerate drug approvals have served as good examples for both the industry and the patient community. We should not omit science and data from advocacy campaigns. R&D should remain a scientific process, not one driven solely by popular vote. However, placing patients at the center of the drug development process can help spur changes and direct attention to unmet medical needs.

We founded a biotech company that aims to address a deadly disease with innovative, transparent research and accelerated therapy development. Our for-profit status allows us to apply general business rules to attract capital and advance our mission. We still face the boundaries obstructing fast development tracks, but this generation of patients, "patients 3.0," will eventually break down traditional boundaries and help streamline and expedite drug development. With more than 50, 000 people diagnosed with ALS each year, the sooner, the better.

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Theme 1 Clinical Management

P1 CLINICAL DIAGNOSIS AND MULTIDISCIPLINARY MANAGEMENT IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Keywords: nutritional status, deglution, NIV

Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by a loss of motor neurons in the cerebral cortex, brainstem and spinal cord, leading to skeletal muscle atrophy, paralysis and death. The clinical onset can be bulbar, affecting swallowing and phonation, or spinal, presenting as skeletal muscle atrophy. Dysphagia is a condition that leads to decreased food intake and body mass, malnutrition and dehydration. Weight and muscle loss are related to the disease progression. The changes in respiratory function may negatively affect the quality of life and the survival of ALS patients. For this reason, one multi-professional program was implemented in order to classify patients at different stages of the disease.

Objective: To present clinical conditions of the patients attending ALS multidisciplinary teams.

Methods: This is a descriptive study with semi-structured interviews applied for three weeks in some patients, accompanied by a multidisciplinary team of Neuromuscular Disease Research. The evaluation included: anamnesis; assessment of the food consistency; dysphagia; nutritional status; use of non-invasive ventilation (NIV); Speech Intelligibility Scale; and functionality scale (ALSFRS-R).

Results: There were 46 patients evaluated (65.21% male and 34.79% female), 37-79 years old (median 59 years). 39 patients (84.7%) had appendicular involvement (69.2% men and 30.8% women): 24 patients (61.5%) with initial commitment of upper limbs; 11 (28.2%) with lower commitment. Seven patients (15.3%) had bulbar involvement (57.1% women and 42.9% men). The median elapsed time between the first symptoms and diagnosis was 12 months (1-118). Co-morbidity was observed in 11 (23.9%) patients, all with hypertension. 23.9% of patients had been taking some kind of vitamin complex. Dysphagia was observed in 76% of patients and 46.9% had been using non-invasive mechanical ventilation. An alternative way of feeding was found in 33% of cases, with the majority (73%) with GEP. The average BMI was 25.09 Kg/m2 (19 - 31). The average of Score Speech Intelligibility Scale was 6 (1-10); ALSFRS score was 25 (6 - 44).

Discussion and conclusion: A multidisciplinary palliative approach can prolong survival and maintain quality of life of ALS patients. Multiple problems require a multidisciplinary approach including rehabilitation to maintain motor function, nutritional support (enteric feeding, gastrostomy), respiratory support, augmentative communication devices and palliative care. The evaluation systematically helps in controlling the symptoms and facilitates early intervention so as to minimize the evolution of disease.

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P2 EVALUATION OF THE APPLICATION OF THE EUROPEAN GUIDELINES FOR DIAGNOSIS AND CLINICAL CARE OF ALS PATIENTS

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Keywords: EAN recommandations, Health Care Quality, Evaluation

Objective: To evaluate the degree of application of the recommendations edited by the European Academy of Neurology (EAN)/European Federation of Neurological

Sciences (EFNS) in 2005, on the multidisciplinary management of amyotrophic lateral sclerosis (ALS) in clinical practice.

Methods: Multicenter observational study involving six French ALS-referral centers recruiting prevalent and incident cases. Recommendations were translated into ad-hoc questions referring to key steps in the management. The application was evaluated by an independent clinical research assistant who examined the medical charts (MCs). When needed, an independent board-certified neurologist answered the questions based on the examination of MCs and interview of the caring neurologist. Questions regarding diagnosis and communication were asked to patients through a self-administered questionnaire.

Results: We included 376 patients (176 incident, 200 prevalent cases; median age at diagnosis: 62.8 years (IQR 55.7-72.3); sex-ratio: 1.37; 27.3% of bulbar onset). All the topics covered in the recommendations were evaluated: diagnostic delay (eg mean 13.6 months, associated with age and onset); breaking the news (eg in more than 90%, satisfaction of criteria for communication quality); multidisciplinary and sustained support (eg in 90%, clinic visits scheduled at every 2-3 months); riluzole offering; symptom management; genetic testing; ventilation; communication defects; enteral nutrition; palliative and end-of-life care. We also identified characteristics associated with poor compliance with some guidelines (schedule of visits, delay of riluzole initiation).

Conclusion: This is the first evaluation of the application of the EAN/EFNS recommendations for the management of ALS. This allows us to propose sources of improvement for caring neurologists.

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P3 REVISITING EARLY DIAGNOSIS IN ALS

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Keywords: diagnosis, biomarker, meta-analysis

Objectives: The currently utilized diagnostic Awaji criteria in Amyotrophic Lateral Sclerosis (ALS) can result in a diagnostic delay of up to ten months. Subsequently we utilized a three-pronged approach, initially an individual patient data (IPD) meta-analysis was undertaken to evaluate the diagnostic utility of the Awaji criteria. We then performed the first prospective multicenter study looking at the current diagnostic criteria. Finally we looked at the addition of a novel transcranial magnetic stimulation (TMS) technique as an objective biomarker of upper motor neuron dysfunction, to further improve the current criteria.

Methods: An IPD meta-analysis was performed after communicating with key researchers and authors who published diagnostic studies looking at the Awaji criteria. In total IPD was available for 1086 individuals across 8 published studies. Statistical modelling was undertaken using SAS (9.3). The first prospective multicenter study looking at the Awaji criteria was undertaken in Sydney, Australia, according to the STARD criteria in 369 patients. Finally, a prospective multicenter study was undertaken looking at the diagnostic utility of threshold tracking (TMS) in 333 patients.

Results: An IPD meta analysis of 1086 individuals revealed that the current Awaji criteria were more sensitive than the revised El Escorial criteria (rEEC) in all, except one study (7/8 studies). The Awaji criteria were more robust than the rEEC criteria (Sensitivity Awaji 70%, rEEC 45%, $P<0.0001$). The Awaji criteria performed better than the rEEC in both bulbar and limb onset disease ($P<0.0001$). In the multicenter prospective study, the Awaji criteria were compared in a cohort of 369 patients. Specificity was 100% across both criteria. Sensitivity was 57% with the Awaji and 39% with the rEEC criteria. Finally in the TMS prospective study, by objectively measuring evidence of UMN dysfunction, an extra 34% of patients could be diagnosed at the first visit. If TMS changes of cortical dysfunction were added to the current Awaji criteria, the sensitivity of the Awaji criteria could be increased to 86%. Furthermore, 88% of Awaji 'Possible' patients could be reclassified as 'Probable/Definite'.

Conclusion: Whilst an individual patient data meta-analysis revealed a better sensitivity for the Awaji criteria when compared to the older rEEC criteria, there were still a number of patients that could not be classified early. Furthermore all the studies that have looked at the Awaji criteria have been single centre in design, and generally retrospective in nature. The first prospective multicenter study looking at the Awaji criteria, revealed that the sensitivity of the Awaji criteria was lower than expected, most likely related to the inclusion of patients with early onset disease. Finally by utilizing an objective neurophysiological marker of UMN dysfunction, the diagnosis of ALS could be made earlier, which will then hopefully translate into earlier recruitment into clinical trials, ideally within the critical time frame for therapeutic response.

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P4 BREAKING THE NEWS OF AN MND DIAGNOSIS: A SURVEY OF NEUROLOGISTS IN AUSTRALIA

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Keywords: training and education needs of neurologists, communication skills, best practice protocols

Background: Communicating the diagnosis of MND is daunting for both neurologists and patients. The manner the patient receives the diagnosis is acknowledged to be the first and one of the most sensitive steps in palliative care.

Objective: To establish a knowledge base of usual practice of breaking the news of an MND diagnosis in Australia, and to highlight differences and similarities in Australian practice compared to international practice guidelines.

Method: This study consisted of an anonymous postal survey facilitated by all MND Associations in Australia and the peak body of neurologists, in 2014. Questions centred on the SPIKES protocol for communicating bad news, how patients' consultations were conducted, personal experiences in giving the diagnosis, the communication plan and support for patients, the neurologists' education and training needs.

Results: Completed postal surveys were received from 73 neurologists (50% response rate though estimated to be 80% of all those who deal with MND in Australia). Nearly 70% of neurologists reported finding it "somewhat to very difficult" communicating the MND diagnosis, and 65% reported feeling moderate to high stress and anxiety at the delivery of diagnosis. This national study has detailed where Australian practices in breaking the news of an MND diagnosis are in line with European guidelines and where there is room for improvement such as in the length of consultation, the period of follow up and referral to MND associations. Two thirds of responding neurologists were interested in both further training responding to patient's emotions and development of best practice guidelines. With nearly 40% of people with MND dissatisfied with their experience of the diagnosis delivery (comparison from a parallel survey), there is room for improvement in the practice of neurologists in Australia in several aspects.

Conclusions: This is the first national study to provide a comprehensive insight into the process of delivering the MND diagnosis from the neurologists' perspective. Improvements can be attainable through educational and training programs aimed at neurologists and neurology trainees to improve their skills in responding to patients' emotions, and the development of a best practice protocol in communicating the MND diagnosis, based on the evidence from this study and existing international protocols.

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P5 FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS – WHAT MOTIVATES FIRST-DEGREE RELATIVES TO PARTICIPATE IN A PRECLINICAL STUDY AND HOW IS THE STUDY PROCESS PERCEIVED?

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Keywords: presymptomatic, familial ALS

Background: Most forms of familial amyotrophic lateral sclerosis (ALS) are autosomal-dominantly inherited. The risk for first-degree relatives to inherit the gene mutation is 50%. In an ongoing longitudinal cohort study (GPS-ALS Study) people at risk for familial ALS are phenotyped with a special focus on biomarkers, energy metabolism, neuropsychological assessment, neuro-imaging and clinical neurological symptoms.

Objectives: The gene status was not revealed to the study participants or the investigators. Since the study creates a complex ethical situation of examining persons who might face a fatal neurodegenerative disease, we were prompted to examine the broader effects the study process and dealing with the potential disease has on the participants.

Methods: A structural phone interview was performed a week after the examination days by a staff member. Different parameters were explored such as motivational aspects, study process, side effects of the lumbar puncture and changes in attitude towards ALS and the risk of ALS. Finally the participants were asked about their personal estimation of their gene status on a percent scale and given time to share their thoughts in an open question.

Results: Of the first 32 subjects recruited: 27 were available for a phone interview; 40.6% were male and 59.4% female; 62.5% were gene negative and 37.5% positive.

The most important motivation aspects were: 1) contributing to scientific progress (mean=9.19); 2) help ALS-patients in general (mean=8.93); 3) for relatives (mean=8.78). Personal curiosity as a motivation was rated lowest with a mean of 4.74. No significant gender differences were detectable.

In estimating the own gene status, 31.3% did not have a tendency at all, rating a 50% chance. 15.6% estimated their chance to be a carrier to be lower than 50% and 15.7% supposed it is more than 50% likely. 31.3% did not want to answer the question and 3.1% already knew their gene status.

Perception of the study process was evaluated. The mean value on the numeric rating scale (wearing to pleasant) was 7.37. The given information was perceived more informative than confusing (mean=8.78) and the average evaluation of the information being rather disturbing (0) or calming (10) was 6.26.

A lumbar puncture was performed in 53.1% of the subjects, with 35% of these reported post punctional headache.

Conclusions: The GPS-ALS study was well accepted. Most subjects were satisfied with the study process and did not regret participating. Being able to contribute to ALS research was very important to all participants. However, it should be kept in mind that it is possible that subjects, who were dissatisfied with the study process, may have been unavailable for the phone interview.

These results suggest that the opportunity to participate in preclinical research offers some intrinsic benefits to risk carriers.

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P6 THE SPECTRUM OF CLINICAL OPINION ON GENETIC TESTING IN ALS

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Keywords: genetic testing, familial ALS, presymptomatic

Background: As genetic technologies improve, the number of genes implicated in ALS increases and it is clear that sporadic ALS (SALS) has a strong heritable component, mediated in part by genes known to cause familial ALS (FALS). Nevertheless, genetic testing is often only offered to patients with a clear family history of the disease and variable penetrance, along with unknown pathogenicity for some genes, casts doubt on the utility of genetic testing in some cases.

Methods: To investigate the opinions of ALS specialists towards genetic testing, we surveyed 167 neurologists and other specialists regarding their definition of FALS and genetic testing in FALS, SALS and presymptomatic family members.

Results: The majority of those surveyed (73.3%) did not consider that there is uniform definition of FALS. 57.5% consider a family history of FTD in their definition of FALS. The majority of respondents (90.2%) offer diagnostic testing for patients meeting their definition of FALS, compared to 49.4% who offer it to those with SALS. A substantial proportion of respondents (48.5%) consider testing positive for a known ALS gene to be sufficient to meet the criteria for FALS. Although there was some regional variation in the gene panel tested by respondents, there was a general consensus for the prioritization of four main ALS genes: *SOD1*, *C9orf72*, *TARDBP* and *FUS*, with relatively few respondents claiming that they test for the other genes included in the survey.

There was significant regional variation in whether respondents would offer presymptomatic testing to ALS families ($p = 1.27 \times 10^{-10}$), and whether they would seek testing themselves were they to belong to an ALS kindred ($p = 2 \times 10^{-10}$).

Conclusions: There is an urgent need for consensus in the definition of Familial ALS, and for guidelines for genetic testing.

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P7 THE STRUCTURE AND USE OF THE AMYOTROPHIC LATERAL SCLEROSIS FUNCTIONAL RATING SCALE REVISED

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Keywords: ALSFRS-R, Rasch, outcome measures

Background: Franchignoni and colleagues have recently shown that the Italian adaptation of the Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) is multidimensional and should, as a consequence, be reported as a profile of Bulbar, Motor Function and Respiratory domains, rather than a total score (1).

Objectives: Given the widespread use of this scale in motor neurone disease (MND) research, confirmation of these findings is urgently required, and the potential use as a profile illustrated.

Methods: TONiC is an ongoing, longitudinal, multicentre study in the UK which invites people with MND to complete a questionnaire pack containing a variety of measures, including the Neurological Fatigue Index-MND (NFI-MND) and demographic data. Clinicians provided the ALSFRS-R. Data from these scales were examined by Rasch analysis.

Results: 178 patients had returned complete baseline questionnaires by the end of 2014. Their mean age was 65.6 years (SD 11.0) and mean duration of disease was 2.7 years (SD 3.3). 61.2% were male. Rasch analysis of the 12 item total score confirmed multidimensionality (ideal t-tests <5%; actual 26%), and considerable misfit to the model (Chi-Square 66.3 (df 24) $p<0.001$). In contrast, each of the three domains: Bulbar, Motor Function and Respiratory, satisfied model expectations (Chi-Square $p > 0.05$); were all unidimensional (t-test <5%); and displayed no Differential Item Functioning by age, gender, duration, marital status or religious beliefs. Nevertheless category ordering of items remained problematic, and showed a lack of monotonicity.

Taking the mid-point of each domain scale range as a simple cut between 'poor' and 'good' function of the domain, with a high score being 'good', patterns of impact can be determined. 51% of patients in the current study had low impact, 32.6% rated poor on one domain; 13.5% on two domains, and 2.8% on all three domains. There was no significant difference for age or duration across the impact groups (ANOVA $p > 0.05$), or by gender (Chi-Square $p > 0.05$). However, strong gradients were observed across health status measures, including fatigue (NFI-MND) (Kruskall-Wallis Chi-Square $p < 0.001$).

Discussions and conclusion: The ALSFRS-R is confirmed to be multidimensional, and should be used as three distinct profiles, each of which has been shown to provide satisfactory fit to the Rasch model. Consequently the raw score of each domain is a sufficient statistic, and the domains can provide different ways of profiling patients. Further work should be undertaken on reviewing the category ordering of some items.

Acknowledgements: We thank the patients for their participation, and the MND Association (UK), NIHR and Walton Neuroscience Charity for support.

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P8 A PROPOSED CURRICULUM FOR MOTOR NEURONE DISEASE SPECIALIST NURSES

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Keywords: Education, Curriculum, Nursing

Background: In the United Kingdom, there is approximately one neurologist for every 150,000 population compared to one per 25,000 in Europe (1). The role of specialist nurses has been developed to meet the care needs of MND patients due to the current workforce constraints. To date, no MND specific curriculum exists for specialist nurses despite similar curricula being available in Epilepsy and Parkinson's disease (2). Locally, we initiated curriculum development when a new MND nurse was appointed and a need for a structured training programme was identified.

Objectives: Our objective was to design a clear, practically based, patient focussed curriculum for specialist MND nurses. A new curriculum would allow for varying levels of competency and specialism at entry level producing a spectrum of staff from nurses with a special interest in MND to independent MND Nurse Practitioners.

Methods: Prior to designing a curriculum, a situational analysis was performed to review factors relevant to our organisation (3). This analysis involved an in depth review of the current training pathway in our organisation through semi structured interviews with those who had both received and delivered training. External factors such as the expectations of patients and employers were considered alongside internal factors such as personal learning goals. Similar speciality nursing frameworks for neurological conditions such as Epilepsy, MS and Parkinson's disease were also evaluated.

Results: We chose a spiral curriculum design model to ensure that core concepts could be revisited as individuals progressed through training. To align with other neurological curricula, three levels of competency were selected; competent, specialist and highly specialist. The core components of the curriculum are outlined below:

Aetiology, pathology, diagnostics and assessment; Providing support and advice to patients and carers; Symptom management; Medicines management; Managing through the stages of MND including end of life care; MDT and liaison working including with charity organisations (MND Association) and national centres; Education and audit; Accountability

New trainee nurses could work through the above topics initially developing competency and moving on to highly specialist skills. Furthermore, the competency levels could be used to differentiate nurses with a special interest from independent practitioners.

Discussion: There is an urgent need to formalise a training curriculum for MND specialist nurses. We believe that this model represents a needs based and practical approach to training. We hope that it can be used as a benchmark to develop individuals and drive excellent care delivery in MND.

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P9 ADHERENCE TO THE CLINICAL PRACTICE GUIDELINE FOR PHYSIOTHERAPY TO PATIENTS WITH ALS IN DENMARK: A CROSS-SECTIONAL STUDY

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Keywords: physiotherapy, guideline, implementation

Introduction: Implemented clinical guidelines can build bridges between research and clinical practice. Implementation strategies have to be multi faced and tailored to local barriers, but the nature of the clinical guideline has an important impact on the implementation. A national clinical guideline (NCG) for physiotherapy to patients with ALS was developed as a bottom-up project with high involvement of ALS-physiotherapists, ALS-patients and patient organizations. The NCG consists of 16 recommendations regarding exercise, mobility and chest physiotherapy. All recommendations are based on weak evidence but containing important information for physiotherapists in ALS-teams. The NCG was published at the Danish Clearinghouse in April 2014. A tailored implementation process started in May 2014 targeting the ALS-physiotherapists in the Danish ALS-teams.

Objectives: The aim of the study was to evaluate the use of the NCG for physiotherapy to ALS-patients in the Danish ALS-teams by national chart audits.

Methods: Ten hospitals in Denmark treating in- and outpatients with ALS were eligible for this national cross-sectional study. The implementation strategy was tailored to local barriers. Elements in the strategy were: high involvement of target group; involvement of ALS-patients and patient organization; and educational meetings for ALS-physiotherapists. NCG use was assessed by chart audit in the period January-February 2015. A consensus process indicator was developed in December 2014 for the national audit.

Results: All ten hospitals participated in the implementation interventions. Eight hospitals participated in the evaluation of NCG use. Eighty-six medical records were included in the audit. The consensus process resulted in a broad process indicator. The indicator found that the NCG was used in 98% of the contacts.

Conclusion: The NCG for physiotherapy to patients with ALS is well implemented in the Danish hospitals with ALS-team. The indicator used for chart audit is broad, more specific indicators as a clinical reasoning algorithm could give more value to audits and the quality of physiotherapy to ALS-patients. The study further shows that many barriers for implementation of a NCG, can be incorporated in the developmental process.

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P10 COIN ROTATION TASK FOR ASSESSING MANUAL DEXTERITY IN ALS

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Keywords: ALSFRS-R, prognosis, progression

Background: The Coin Rotation Task (CRT) is an easily administered bedside test that assesses manual dexterity (1). It has been validated in multiple sclerosis (2). Impaired manual dexterity is also frequent in ALS and despite low evidence and site of onset, has a prognostic value (2).

Objectives: The objectives of this study are (a) to establish preliminary normative values, and (b) to evaluate the correlation of the CRT with: (i) the ALSFRS-R total score and manual dexterity questions (handwriting, cutting food, and dressing); (ii) the Box and Blocks Test (BBT); and (iii) a hand held dynamometer.

Methods: For the CRT, a U.S. nickel was rotated with the fingers for 20 times. Time to completion was measured (1). For the BBT, individuals had to move as many wooden cubes as possible, one at a time, from one compartment to another, for one minute (3). Isometric contractions for 3 to 5 seconds were measured with the hand held dynamometer. Both hands were evaluated with the three tests. Twenty-eight ALS patients were drawn from those attending to the Instituto de Ortopedia Infantil Roosevelt (Bogotá - Colombia) between February and April 2015. ALSFRS-R were obtained from all but two patients. The BBT was obtained from all but one patient. The CRT and the dynamometer were obtained from all patients. Thirty-four non-symptomatic individuals served as the reference population. Two patients completed the CRT with the dominant hand but were not able to complete the task with the non-dominant one. Nine patients were not able to complete the CRT with any hand. The time for these patients was defined as 3 times the interquartile range of those who did complete the task. The upper limit of normal was set as 2SD above mean. Patients and controls were compared with the Mann-Whitney U test. Spearman's rho was used for assessing linear dependence between the ALSFRS-R, the BBT, and the dynamometer with the time obtained in the CRT.

Results: The normal values for the CRT are 13.27 (3.2) and 15.34 (3.6) seconds, for the dominant and non-dominant hand, respectively. Both groups were different (Mann-Whitney U test $p<0.001$). Significant linear inverse correlation was found between the ALSFRS-R, the BBT, and the dynamometer with the CRT (Spearman's rho -0.41 to -0.68; p-values 0.03 to 0.0001).

Conclusions: A simple bedside test that assesses dexterity in ALS may aid in the rehabilitative treatment as devices to improve life may be prescribed when deterioration is proved objectively. It also may be useful for assessing progression. Further analyses are mandatory.

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P11 SYSTEMATIC INVESTIGATION ON NEEDS AND USE OF TECHNOLOGICAL DEVICES IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS AND CAREGIVERS: A NATIONWIDE, MULTICENTRE SURVEY

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Keywords: quality of life, techonological devices, multidisciplinary care

Background: ALS is a neurodegenerative disease which causes progressive physical impairment, together with worsening limitations in the functions of breathing, swallowing and communication (1). The whole range of activities of daily living can be severely compromised, causing an increasing need for assistance, and clinical treatments available are not at all curative. Therefore, resources helping patients and their families to adapt to such a life condition are emphasised, in order to maintain patients' and caregivers' quality of life as high as possible. However, since the involvement of caregivers in the support and management of patients is crucial, a systematic investigation on needs and use of technological devices is warranted.

Objective: To explore the unmet needs of available assistive technology in a large population of ALS patients and related caregivers, specifically investigating the level of satisfaction on existing devices and their usefulness in improving the quality of life, in four identified areas (communication, motricity, surveillance and domotics).

Methods: We consecutively enrolled 72 ALS patients and 59 caregivers, followed by three Italian tertiary multidisciplinary ALS centres (Milano, Arenzano, and Messina). The study has been proposed by the Italian Foundation for ALS Research (ARISLA). Questionnaires were focused on patients' basic skills, and for each area patients were asked to point out which was the main aim of the device. Furthermore, symptoms severity, acceptance of the device, expectations, limits and desiderata were investigated. Similar domains were investigated also among caregivers.

Results: 66% of patients and 75% caregivers reported dissatisfaction about devices for communication. The main cause was complexity in the usage. Half of patients and caregivers were dissatisfied about devices for the motor deficit, including upper limb aids (40%), electronic wheelchair (43%), and manual wheelchair (30%). The main cause of dissatisfaction was the lack in autonomous use and level of customization. Few patients and caregivers expressed an opinion on domotic and monitoring devices due to the difficulty in obtaining the devices.

Discussion and conclusion: Basing on the results of the questionnaire, an urgent need of amelioration of primary needs (mainly mobility and communication) is emerged. To meet these requests, it is necessary to improve usability and handiness of the already existing devices implemented on specific ALS patient's features. The results of this work may be important to design and orient future development and implementation on assistive technology and steer charity's funding in this area.

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P12 ASSISTIVE EQUIPMENT USE BY PEOPLE WITH ALS/MND IN AUSTRALIA

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Keywords: equipment utilization, phenotype, multidisciplinary care

Background: People with ALS/MND (pw ALS/MND) benefit from access to assistive equipment to address disability and improve social participation. This equipment ranges from small items such as adaptive cutlery or a foot splint, through to large expensive items such as motorised wheelchairs and electric beds. The pattern of muscle weakness varies between phenotypes, and so it could be expected that the need for specific equipment items also varies by phenotype. Equipment use is further complicated by the passage of time; equipment needs will change and progress over the course of the disease. There are therefore many considerations for therapists attempting to predict and meet equipment needs of pw ALS/MND.

Objectives: This study aimed to examine the relationship between type of equipment prescribed, length of time since onset of symptoms and phenotype differences in equipment use by pw ALS/MND.

Methods: Prospective, longitudinal, observational consecutive cohort study in a multidisciplinary clinical

setting, Melbourne, Australia. The following data was collected by the treating Occupational Therapist, Physiotherapist and/or Speech Therapist at each clinic visit, or on inpatient admission: equipment in use, ALSFRS-R score, date of onset of symptoms and phenotype. Information was collected on use of 56 items of equipment, grouped into eight categories: speech devices; transfer devices; mobility devices; power wheelchairs; orthoses; ADL equipment; assisted technology and home modification equipment.

Results: Data was collected on 273 pw MND, mean age 67 years (SD +/- 11.7), range 36-94 over the period March to December 2014. Phenotype distribution was as follows: ALS Lumbar (33.0%); ALS Cervical (25.6%); ALS Bulbar (22.7%); PLS (8.4%); Flail Arm (6.6%); Flail Leg (1.8%); Primary Muscle Atrophy (1.5%). The median length of time since symptom onset was 24 months, range 2-271 months. The number of equipment items per pw MND ranged from none to 20, (mean 5.3). Equipment use was mapped over time revealing differing trends between phenotypes. Significantly more pw ALS Bulbar (39%) were using assistive technology compared to 9% of ALS Lumbar (CI= 0.15-0.58), whereas significantly more pw ALS Lumbar (30%) were using transfer devices compared to ALS Bulbar (11%) CI=(0.99-0.48). There were some unexpected results, such as pw Flail Arm phenotype were twice as likely to be using an electric bed than those with Flail Leg phenotype.

Discussion and conclusions: This information can be used by therapists to help predict which people with ALS/MND will need certain items of equipment, and when. It will also help Service Providers estimate types and amount of equipment needed to support pw ALS, and potentially, costs associated with this.

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P13 ELECTRONICALLY AUGMENTED TIMED UP AND GO TEST (EATUG) TO EVALUATE MOBILITY AND BALANCE IN AMBULATORY PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS (AMBALS)

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Keywords: balance, mobility, timed up and go(TUG)

Background: The Timed Up and Go (TUG) test is a widely used clinical measure of mobility, balance and fall risk in older adults and in individuals with neurological diseases. The TUG records the timed required for a participant to stand up from a chair (STS), walk 3 meters and turn back to the chair (WT), turn-before-sitting (TS), sit down (SD). Current clinical practice suggests that patients with balance and mobility problems have longer TUG times and are more likely to fall than those with shorter times. How each of the TUG segments relates to the impairment of balance and mobility in ambALS is not currently known.

Objective: To test the feasibility of using a body-fixed Inertial Measurement Unit (IMU), an electronic device that measures velocity, acceleration, and change in position to provide insight into TUG performance in ambALS patients.

Methods: Maximum torso flexion/extension angle (MTFEA in deg), Peak Flexion and Extension Angular Velocity (PFAV/PEAV in deg/sec), Peak Vertical Acceleration (PVA in m/sec2), and Mean/Peak Turning Angular Velocity (MTAV/PTAV in deg/sec) were evaluated during TUG manoeuvre segments in 20 ambALS (16 males, 4 females; age=63 ± 11; ALSFRSR gross motor = 9 ± 2) and 18 ambulatory individuals with Parkinson's disease (ambPD) (15 males, 3 females; age = 65 ± 110; UPDRS III = 19 ± 12). Nine ALS and 4 PD patients had a history of falling. Subjects performed two consecutive TUG tests wearing a chest-mounted IMU. The average of the two trials was used for analysis. Independent t-tests were used to compare the performance of ALS vs. PD in the parameters of each TUG segment. The Pearson correlation coefficient was used to determine the relationship between EATUG and patient clinical measures.

Results: Statistically significant correlation (Pearson correlation coefficient) was observed between EATUG and the ALSFRSR-GM score ($r = -0.584$, $p = 0.007$) and the UPDRS III score ($r = 0.773$, $p = 0.001$) indicating that the EATUG correlated with the clinical status in both groups. There was no difference in total EATUG time between ALS and PD (11 ± 4 and 10.2 ± 2) or the percent of total EATUG time taken to perform each segment between the 2 groups. In the STS segment, the ALS group had higher MTFEA ($p \leq 0.005$), PFAV ($p \leq 0.06$), and PEAV ($p \leq 0.001$).

Conclusions: EATUG evaluating mobility and balance in ambALS and ambPD is abnormal in both groups compared with published control subjects. Maximum torso flexion/extension angle (deg) and Peak Extension Angular Velocity (deg/sec) measured by EATUG significantly differentiates during STS segment in ambALS compared with the disease-control group of ambPD. These observations extend our physiological studies of balance problems in ambALS (1).

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P14 TELEPHONE INTERVIEWS ON RESPITE AND COMMUNICATION RESOURCES TO SUPPORTING HOSPITALS AND PEOPLE WITH ALS IN A PREFECTURE OF JAPAN

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Keywords: respite, communication, telephone interview

Background: It is very important for people at home with ALS to have suitable opportunities for respite and communication support. However, the provision of resources varies considerably between regions. In 2014, we began a mail-in survey to assess the status of respite and communication for patients in Japan with ALS and other intractable neurological disorders.

Objectives: To assess the status of respite and communication for patients in Japan with ALS within a given prefecture, using telephone interviews.

Methods: 16 supporting hospitals were approached to help with this study, 15 people with ALS who were members of the prefectural society of ALS were recruited to complete questionnaires. All participants were informed via post and provided written consent prior to the interview. Between September-2014 and January-2015, telephone interviews were author conducted with IC recording equipment.

Results: There were 15 patients in total, 11 men and 4 women. The mean age was 61 years old, and their total ALSFRS-R scores ranged from 0 to 43, with a mean 16.2. The mean interval from onset to diagnosis was 71.9 months. The interview guide consisted of themes such as awareness, experiences, preparation and utilization of respite and communication support. At the time of interview, 11 were being cared for at home, 3 were receiving hospital care, and 1 was in a facility for the disabled: 7 required no respiratory support, 7 were on TPPV, and 1 was on NPPV. We found that 11 were aware of the word of "respite". Of those, the mean total ALSFRS-R score for patients who experienced respite was 5.4, this was lower than the 21.6 found for those who had never experienced it (p=0.01, t-test).

Meanwhile, hospitals responded regarding their roles: 8 hospitals had provided the established diagnosis; 2 had given any clinical trial; 12 gave second-opinions; 13 offered respite (mainly 7 to 10-days-hospital-stay for each patient's respite); 9 offered some services for acute illness;7 offered long term care; 5 showed coordination of the regional support and 3 offered educational support. In this study, there was no hospital where scheduled respite was provided regularly.

Discussion and conclusions: The prefecture was relatively rural and was somewhat lacking in resources. However, this study supports the conclusion that ALS patients were not fully aware of the term "respite" and that there was considerable variation in the support provided by hospitals to such patients and their families.

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P15 DID MORPHINE USAGE BECOME MORE POPULAR IN JAPAN? BASED ON THE FINDINGS FROM 2015 NATIONWIDE SURVEY

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Keywords: palliative care, nationwide survey, morphine

Background: Although morphine prescription for the ALS patients has not been the standard in Japan, from 2011 Sep 30, morphine for ALS finally became applicable for the national insurance after series of negotiations. We performed nationwide survey again to clarify the changing situation of morphine usage.

Methods: We sent out the survey with 45 questions to 5144 board certified neurologists. We asked about their clinical experiences and thoughts on palliative care. This was the anonymous survey.

Result: 1,391 neurologists returned the questionnaire, and the response rate was 27% (22% in 2012, 34% in 2009). In this paper, we analyzed 1377 as valid responses. The respondents with the experience of prescribing morphine to their ALS patients slightly increased to 35% (32%, 21%). Among physicians who have more than 4 ALS patients of the end of life period, rate of prescribing morphine increased to 72% from 65%. When a neurologist start to prescribe morphine to their patients, most of the respondents answer that they were self taught and self trained, but the ratio of them slightly decreased (45%, 44%, 47.5%). Instead, neurologists who collaborate with palliative physician increased to 28% (22.4%, 17.7%). The main reason why they don't prescribe morphine was because they do not have patients who need to use morphine. This reason increased to 68.5% from 66.7% in 2012 and 65.1% in 2009. On the contrary, the reason

such as the absence of the national insurance coverage decreased to 14.5% from 49.1%, 47.1%. The answer whether one will prescribe morphine in the future was "never" 5.2% (4.7%, 7%), "with insurance application rather than insurance coverage" 22.3% (26.1%, 38%), and "whether or not the insurance application" 67.2% (63.6%, 47%). As shown, many of the respondents became eager to prescribe morphine if they have patient who need it.

Discussion: As a result, the rate of physicians who have experience of using morphine increased slightly as compared to 2012. Likewise, 67% answered that they would use if necessary, and 69% answered the reason of not prescribing morphine was because they did not have appropriate patients. Thus, the majority of neurologists agreed to use morphine on ALS patients. At the same time, 87% of neurologists had less than 3 ALS patients at the end of life period; the neurologists who had more than 4 ALS patients were prescribing morphine more frequently. There is a possibility that the physicians who perform palliative care in ALS are shifted to home physician from neurologists. It might be necessary to research wider target to see the changing situation in the usage of morphine for ALS patients.

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Theme 2 ALS Heterogeneity and Disease Progression

P16 DISTINCTIVE PATHOLOGICAL FEATURES OF THE CU/ZN SUPEROXIDE DISMUTASE 1 (SOD1) MUTATION D102N

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Keywords: SOD1, D102N mutation, SEDI

Background: Amyotrophic lateral sclerosis (ALS) is genetically and pathologically heterogeneous (1). Mapping of genotype-molecular pathologic phenotype associations helps to define the pathophysiological pathways that lead to neurodegeneration. Although SOD1 was the first gene linked to autosomal dominant ALS, and over 180 mutations in SOD1 have been linked with disease, only few neuropathological genotype-phenotype studies have been performed. Here we present a comprehensive assessment of a sibling pair with the D102N mutation, with a focus on p62, TDP-43, FUS, OPTN, UBQLN2 and SOD1 expression. The neuropathology of the SOD1 D102N mutation has not been described in the modern era of neuropathology.

Objectives: To describe the neuropathology associated with the ALS SOD1 D102N mutation in two siblings, with a focus on SOD1 expression and its relationship to other proteins implicated in ALS pathogenesis.

Methods: Immunohistochemistry with antibodies against wild-type and conformation-specific epitopes of SOD1, and against p62, TDP-43, FUS, OPTN, UBQLN2 and neurofilament.

Results: The siblings presented with a pure motor phenotype, which was lower motor neuron dominant. There was no dementia. Age at death was 34 and 42 years. The neuropathology was remarkably consistent between siblings, and, to our knowledge, distinct from other described SOD1 pathologies. Notable features were: 1. restriction of neocortical pathology to the primary mortor cortex; 2. severe loss of lower motor neurons; 3. dominant axodendritic pathology over perinuclear cytoplasmic pathology; 4. distinct white matter aggregate pathology in the cerebellum; 5. Brick-like aggregates in surviving motor neurons, which reacted strongly with conformational SOD1 antibody SEDI (2) and p62 but not FUS or TDP-43; 6. widespread diffuse glial conformation specific SOD1 pathology.

Conclusion: SOD1 D102N neuropathology may be distinct from other SOD1 pathologies including structural appearance of solid aggregates, dominant axodendritic pathology, and specific cerebellar white matter pathology.

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P17 GLOBULAR GLIAL TAUOPATHY (TYPE II) CLINICALLY MIMICKING ALS

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Keywords: GGT, chorea, tau

Background: A new category of 4-repeat (4R) tauopathy designated globular glial tauopathy (GGT) has recently been proposed. GGT is characterized by tau-positive "globular" glial inclusions (GGIs) in oligodendrocytes (GOIs) and astrocytes (GAIs) and exhibits the clinical features of motor neuron disease (MND) and/or frontotemporal dementia (FTD).

Case Study: Here we report a case of GGT Type II in a Japanese patient diagnosed clinically as having ALS with atypical involuntary movement.

A 71-year-old Japanese woman began to exhibit limb weakness and gait disturbance. These symptoms were slowly progressive and accompanied by weight loss. At the age of 74 years, choreic movement appeared in the extremities. On examination, she had muscle weakness and increased deep tendon reflexes in the extremities, with positive Babinski sign in the left leg. Muscle wasting was not evident. Although the appearance of choreic movement is considered to be quite unusual for patients with ALS, a diagnosis of ALS was made.

At the age of 76 years, the patient developed swallowing difficulty, and eventually died of sepsis caused by urinary tract infection about 5 years after disease onset. Dementia was not noted during the course of the illness, and there was no family history of MND, dementia or other neurological disease.

At autopsy, the brain weighed 1,100 g. Atrophy was evident in the frontal lobe, especially in the precentral gyrus. Histologically, severe neuronal loss and gliosis were observed in the motor cortex. Although less severe, such changes were also evident in other brain regions, including the basal ganglia and substantia nigra. In the spinal cord, severe degeneration was observed in the corticospinal tracts, and neuronal loss and gliosis were comparatively mild in the anterior horns. No Bunina bodies or TDP-43 inclusions were found. Immunostaining using antibodies against hyperphosphorylated tau (AT8) and 4R tau (RD4) revealed numerous tau lesions in the motor cortex and subcortical white matter, as well as in the pyramidal tracts. In the affected areas, morphologically unique glial tau lesions termed GOIs and GAIs were a characteristic feature. GGT has now been classified into three types.

Discussion and conclusion: We previously reported cases of GGT Type III in which frontotemporal lobar degeneration, MND and parkinsonism, and predominant occurrence of GAIs, were characteristic. The present case was considered to be an example of GGT Type II (pyramidal features reflecting motor cortex involvement and corticospinal tract degeneration). Although extrapyramidal features can be present in GGT Types II and III, choreic movement appears to be very rare. Pathologically, in the present case, predominant occurrence of oligodendrocytic tau lesions, including GOIs, was a feature.

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P18 GLOBULAR GLIAL MIXED FOUR REPEAT TAU AND TDP-43 PROTEINOPATHY WITH MOTOR NEURON DISEASE AND FRONTOTEMPORAL DEMENTIA

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Keywords: frontotemporal lobar degeneration, tau, TDP-43

Case Study: Amyotrophic lateral sclerosis (ALS) may be accompanied by frontotemporal dementia (FTD). We report a case of glial mixed tau and TDP-43 proteinopathies in a patient diagnosed clinically as having ALS-D. A 76-year-old Japanese woman became aware of gait disturbance, and subsequently developed dysarthria. On examination, she showed atrophy and fasciculation in the tongue, a hypoactive gag reflex, and muscle weakness in the upper extremities. Increased deep tendon reflexes were also present in the upper and lower extremities, with positive Babinski sign in both legs. About 10 months after onset, at the age of 77, she was diagnosed as having ALS. Her mental performance deteriorated rapidly and a state of apathy ensued; at this stage, the clinical diagnosis of ALS-D was made. She also suffered from progressive respiratory distress and underwent tracheotomy for artificial respiratory support.

At the age of 78 years, she eventually became bedridden in a totally locked-in state. Brain CT scan performed at the age of 81 years revealed frontotemporal atrophy. At the age of 85 years, the patient died of septic acute cholecystitis, about 9 years after onset of the disease. There were no parkinsonian features during the disease course. There had been no family history of ALS, dementia or other neurological disease.

A general autopsy was performed, at which time the brain weighed 910 g. Histologically, loss of lower motor neurons and degeneration of the pyramidal tracts were evident in the spinal cord and brainstem. The brain showed frontotemporal lobar degeneration (FTLD); the most severe neuronal loss and gliosis being evident in the precentral gyrus. Although less severe, such changes were also observed in other brain regions, including the basal ganglia and substantia nigra. AT8 immunostaining revealed that predominant occurrence of astrocytic tau lesions termed globular astrocytic inclusions (GAIs) was a feature of the affected regions. These GAIs were Gallyas-Braak negative. Neuronal and oligodendrocytic tau lesions were comparatively scarce. pS409/410 immunostaining also revealed similar neuronal and glial TDP-43 lesions. Interestingly, occasional co-localization of tau and TDP-43 was evident in the GAIs. Immunoblot analyses revealed band patterns characteristic of a 4-repeat (4R) tauopathy, corticobasal degeneration and a TDP-43 proteinopathy, ALS/FTLD-TDP Type B. No mutations were found in the MAPT or TDP-43 genes.

We consider that this patient harbored a distinct, sporadic globular glial mixed 4R tau and TDP-43 proteinopathy associated with motor neuron disease and FTD.

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P19 TAU OLIGOMER ACCUMULATION IN PATIENTS WITH GLOBULAR GLIAL TAUOPATHY (GGT) TYPE III

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Keywords: globular glial tauopathy, tau oligomer, 4-repeat tau

Background: Globular glial tauopathies (GGT) comprise a group of rare four-repeat (4R) tauopathies characterized by the presence of glial globular tau inclusions, ie, globular oligodendrocytic inclusions (GOIs) and globular astrocytic inclusions (GAIs); GGT exhibit the clinical features of motor neuron disease (MND) and/or frontotemporal dementia (FTD).

We previously reported a sporadic 4R tauopathy in three Japanese patients presenting with frontotemopral lobar degeneration (FTLD), Parkinsonism and MND, in whom predominant occurrence of morphologically unique astrocytic tau (AT8)-positive, Gallyas-Braak (G-B)-negative lesions, which have now been termed GAIs, was a feature in the affected gray matter. These three cases have since been classified as GGT Type III.

Methods: In the present study, we examined the brains of the three cases of GGT Type III: a 62-year-old man with a disease duration of 4 years; a 71-year-old woman with a disease duration of 6 years; and a 49-year-old man with a disease duration of 5.5 years, using an anti-tau oligomer-specific polyclonal antibody, T22 (Merck Millipore, Darmstadt, Germany). A double-label immunofluorescence study was also performed on sections obtained from the motor cortex using rabbit polyclonal T22 and mouse monoclonal AT8.

Results: AT8 and T22 immunostaining clearly depicted neuronal and glial tau lesions in many brain regions, including the motor cortex, which exhibited the most severe neuronal loss. In most of the areas examined, the pathological tau burden recognized by T22 was almost the same as that recognized by AT8. Predominant occurrence of GAIs was also confirmed with T22; neuronal and oligodendrocytic tau lesions were also visible with both antibodies. Double-labelling immunofluorescence indicated that co-localization of hyperphosphorylated tau (AT8) and tau oligomer (T22) was a feature of the tau lesions in many brain regions, including the motor cortex. Immunoblot analysis of PBS-soluble fractions of the affected brain tissues using T22 showed prominent bands at approximately 160 kDa.

Discussion and conclusion: In the present study, of great importance was that in three cases of GGT Type III, immunostaining with T22 demonstrated as many neuronal and glial tau lesions as those revealed by AT8. In a series of studies, we confirmed that tufted astrocytes (TAs) in progressive supranuclear palsy (PSP) and astrocytic plaques (APs) in corticobasal degeneration (CBD) were clearly visible with AT8 and by the G-B method. However, such astrocytic tau lesions recognizable with T22 were comparatively small in number and found in restricted brain regions, such as the motor cortex. On the basis of the available facts, we consider that GTT are different from other 4R tauopathies, such as PSP and CBD, not only in their pathological picture but also in their constituent tau species, and that similarly, GAIs are different from TAs and APs

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P20 ELUCIDATING THE POTENTIAL ROLE OF ANTECEDENT DISEASE IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: pre-existing disease, epidemiology, survival analysis

Background: Recent studies suggest antecedent disease (ie, other disease present prior to ALS onset) could impact the pathophysiology of ALS. We perform a case-control study to examine the prevalence of 11 antecedent diseases in ALS. Additionally, we examine whether each disease, individually or in combination, affect ALS onset age or survival length.

Methods: Prevalence of antecedent disease in a 1288 patient ALS population (Emory University ALS Clinic, Atlanta, GA, USA) is compared to an age, gender, and geography-matched 7561 subject control population using a statistical odds-ratio (OR) with 95% confidence interval. Chi-square analysis and ordinal logistical regression were used to examine impact of antecedent disease on ALS onset age and survival length compared to an ALS population without antecedent disease; Kaplan Meir analysis was also utilized to examine survival length.

Results: Association of ALS with odds of arthritis (OR=0.14); non-ALS neurological disease (OR=0.14); liver disease (OR=0.19); chronic obstructive pulmonary disease or COPD (OR=0.23); kidney disease (OR=0.32); adult asthma (OR=0.39); diabetes (OR=0.47); hypertension (OR=0.56); obesity (OR=0.6); hyperlipidemia or hypercholesterolemia (OR=0.62); and thyroid disease (OR=0.78). All patients with antecedent conditions had an older ALS onset age ($p<0.05$) except those with obesity, kidney disease, or liver disease. ALS patients with antecedent hypertension, diabetes, obesity, arthritis, or kidney disease had a shorter disease duration ($p<0.05$) than the ALS population without antecedent disease; however, logistical regression revealed that shorter disease duration was tied more closely to the older onset age than to the antecedent diseases, themselves.

Discussion and conclusion: The prevalence of antecedent disease was overall substantially less in the ALS population compared to the control population. Most antecedent diseases correlated with a later ALS onset age. We present two potential lines of inquiry to explain our results: 1) Other disease as ALS protection - antecedent diseases infer biochemical neuroprotection to ALS. For example, our results reveal that liver disease is practically mutually exclusive with ALS, and Kaplan-Meir survival curves hint to the possibility that liver disease could positively impact ALS survival length; 2) ALS as other disease protection - the underpinnings of ALS could infer protection to other diseases, possibly via the mechanism hypervigilant regulation or 'too-high' regulatory feedback gains.

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P21 SOD1 MUTATION MIMICS A DISTAL HEREDITARY MOTOR NEUROPATHY

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Keywords: SOD1 mutation, distal hereditary motor neuropathy, phenotype

Background: Distal hereditary motor neuropathy is a heterogeneous group of neuromuscular disorders caused by anterior horn cell degeneration; it is characterized by progressive distal motor weakness and muscular atrophy without sensory impairment.

Case Study: A 70-yrs old woman came to our attention with a 12-year story of slowly progressive distal hyposthenia, wasting and weakness - first on right hand, then right leg and left limbs. At neurological examination, no upper motor neuron signs or bulbar impairment, either sensitive or impairmentwere present. No family history of neuromuscular disorders was reported. Electrophysiological examinations were consistent with second motor neuron damage. Motor evoked potentials demonstrated a four limb upper motor neuron involvement.

Genetic analysis revealed a p.E121G heterozygous missense mutation of SOD1 gene, leading to a substitution of glutamic acid with glycine. Mutations in SOD1 represent 12-23% of familial amyotrophic lateral sclerosis (ALS), but can also be found in 2-7% of sporadic cases, presenting with great phenotype variability, although a more typical limb form is mostly expected, with 3-5 years survival.

Discussion and conclusion: We report an apparently sporadic ALS case carrying a p.E121G heterozygous mutation of SOD1 gene, with a long disease course and prevalent lower motor neuron involvement, clinically resembling a distal hereditary motor neuropathy. This mutation has been recently described in two reports associated with a slowly progressive form of ALS. This report therefore confirms the pathogenic role of the p.E121G mutation as associated with a mild disease phenotype.

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P22 CONCURRENT MOTOR NEURON DISEASE AND SPORADIC PARKINSONS DISEASE: TWO CLINICAL CASES OF BRIGHT-FAHN-SCHWARTZ COMPLEX. FUNCTIONAL ASSESSMENT AND CYTOKINE ASSAYS

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Keywords: Parkinsons disease, cytokines, neuro-inflammation

Background: Recently, oxidative stress and neuroinflammation have been hypothesized to play a key role in the pathogenesis of many neurodegenerative conditions, such as Motor Neuron Disease (MND) and Parkinson's Disease (PD). MND and PD are rarely associated, being observed in only 5-17% of MND patients in the so-called Bright-Fahn-Schwartz complex.

Objectives: To correlate clinical and functional data to blood parameters.

Case study: Two subjects with both MND and PD are described. Both patients were followed up for a 4-year period by means of clinimetric scales (such as Amyotrophic Lateral Sclerosis Functional Rating Scale, ALS-FRS and Unified Parkinson's Disease Rating Scale, UPDRS). Cytokines, selectins, lymphocyte phenotypes, and oxidative and inflammatory markers were assayed every 3 months.

Results: In the older patient (77 years), an isolated swallowing deficit for fluids was observed in 2008. One year later, clumsiness for fine movements was also reported. He also had a family history of Von Recklinghausen Syndrome. The younger patient (63 years), after a 20-year history of lower limb muscle cramps, presented occasional dysphagia, and increasingly frequent falls due to worsening of hypostenia associated

with micrographia. Dyskinesias at lower limbs during sleep and fasciculations were reported later.

Both the patients showed a progressive enhancement of IL-8, RANTES, IGF-1, PDGF, ICAM-1, CD-8, CD-16/56, Reactive Oxygen Species, CD-40 Receptor (CD-40R), as well as a reduction of Total Anti-Oxidants (TAO) and Glutathione Reductase activity. In the older patient, elevated levels of IFN-β, TNF-α Receptor-II, VEGF, and Matrix Metalloproteinase-9 were also reported. The discrepancy between values of biochemical parameters in these patients and normal range levels went progressively increasing during the follow-up period. In particular, Prolactin (PRL), CD-4, CD-8 and CD16/56 mean values significantly ($p<0.01$) correlated with ALS-FRS, whereas CD-4 and CD-40R with the UPDRS scores. When examined diachronically, IFN-β, ICAM-1, CD-40R, CD-4, and CD-19 showed a significant ($p<0.01$) time-related variation, where CD-4 and CD-40R changed inversely and directly proportional, respectively, to UPDRS score progression; RANTES and TAO levels decreased, paralleling the ALS-FRS scores, with the progressive elevation of PDGF, VEGF, Superoxide Dismutase-1 activity, CD-4, CD-8, PRL ($p<0.01$).

Conclusion: These results confirm the important role played by oxidative stress and inflammatory cytokines in the pathogenesis of both MND and PD. If confirmed in a larger sample, they might suggest possible pathogenic mechanisms and therapeutic targets.

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P23 THE ALS STRATIFICATION PRIZE - USING THE POWER OF BIG DATA AND CROWDSOURCING FOR CATALYZING BREAKTHROUGHS IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: stratification, disease model, disease progression

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease with significant heterogeneity in its progression. In order to address this heterogeneity and spur ALS research, clinical care and drug development we need sufficient clinical data and suitable analysis approaches.

Therefore, we developed the Pooled Resource Open-access ALS Clinical Trials (PRO-ACT) platform. The PRO-ACT database currently includes demographics, family history, vital signs, clinical assessment, lab-based, treatment arm, and survival information from 8600 ALS patients. The database was launched open access on December 2012, and since then over 450 researchers have requested the data.

In 2012, we used this data to launch the DREAM ALS Prediction Prize4Life, a crowdsourcing challenge seeking the development of more accurate tools for estimating disease progression at the individual patient level. In a simulation, the winning algorithms could reduce the number of patients needed for future clinical trials by 20%. The best performing methods also predicted disease progression consistently better than a group of world leading clinicians. Finally, the algorithms uncovered several novel predictors of disease progressions that can shed light on the mechanisms behind the disease. The algorithms are now being used by several clinical trials and clinics.

In summer 2015, using the existing PRO-ACT database as well as new clinical trial data to be integrated into the PRO-ACT database in 2015, we are launching the DREAM ALS Stratification Prize4Life challenge. The ALS Stratification challenge aims to address directly the problem of ALS patient heterogeneity with regards to important clinical targets such as ALSFRS progression and survival. In the challenge, we ask participants to derive meaningful subgroups of ALS patients along with the clinical features to characterize them. The algorithms that perform best in the ALS Stratification Challenge will be declared late 2015, including the features separating different patient sub groups and implications for clinical trial planning.

These results demonstrate the value of large datasets and crowdsourcing challenges not just for developing a better understanding of ALS natural history, prognostic factors and disease variables, but also for providing new tools that aid clinicians in the improvement of clinical trials as well as patient care.

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P24 CHARACTERISTICS ASSOCIATED WITH DECLINE OF ANTHROPOMETRIC MEASUREMENTS IN ALS PATIENTS

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Keywords: body mass index, nutritional status, antrophometry

Background: The changes of body composition are an independent prognostic factor in ALS. Body weight reduction may accelerate the neuronal degeneration. Anthropometric measurements, suitable for assessment of nutritional status, allow early diagnosis and preventive measures.

Objective: To investigate the characteristics associated with decline of anthropometric measurements.

Methods: We evaluated 53 patients in the first medical. Nutritional evaluation was performed according to BMI and anthropometric measurements of the arm: arm circumference (AC); triceps skinfold (TS); bíceps skinfold (BS); sub cutaneous fold scapular (SFS); skinfold above sacroiliac (SS); muscular arm circumference (MAC); and arm muscle area (AMA).

Results: The study comprised of mostly men (58.5%), aged 55.8 years, with 36% over age of 60 years. Appendicular ALS composed the majority of ALS cases (79.2%) and the time between onset of symptoms and definitive diagnosis was 360 days. The median score observed in ALSFRS was 33 points (13-44). Only 3.8% of patients presented with a low weight.

We observed a decline in the measurements of BS, SS, SFS and MAC in 50% patients. For BMI, AC and skinfolds, a significant variation in relation to the initial period was demonstrated. Bulbar ALS and being overweight at the start of the study showed a greater decline in body mass. Age and onset of symptoms were not associated to greater adipose reserves decline. Finally, a low ALSFRS score and diagnosis confirmed after 12 months showed greater decline in triceps skinfold.

Conclusions: Nutritional evaluation according to anthropometry of arm and BMI are sensitive to measure changes in body composition of ALS patients with early disease.

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P25 CORRELATION BETWEEN PROGRESSION RATE, NEED FOR EXTERNAL AIDS AND SURVIVAL IN PATIENTS WITH ALS

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Keywords: progression rate, external aids, survival

Background: The progressive course of ALS confronted us with patients with ever-changing care needs. Establishing the correlation between progression rate with the need of external aids and the survival of patients with ALS, can improve clinical prediction and determine the time to establish the appropriate therapeutic interventions.

Objective: Determine the correlation between the progression rate with the need for external aids and survival of patients with ALS.

Methods: From our database of ALS in the Instituto de Ortopedia Infantil Roosevelt (Bogotá-Colombia), we identified patients who fulfilled the criteria of possible, probable or definite ALS according to the Awaji criteria, with tracking since March 2009 until March 2015, with an average interval of 15 months (SD 9) between the first and the second assessment. In all patients, in the initial assessment recorded, the date of onset of symptoms and the total score of the ALSFRS R with that, established the progression rate of the disease defined as the ratio between 48 - ALSFRS R score at first assessment/months of evolution.

In the second assessment the need for external aids was recorded such as: nursing; oxygen; BPAP/CPAP; gastrostomy; wheelchair; people for transfers; diaper; and communication devices. We measured the total external aids score with an ordinal scale (0-8) based on the presence/absence (0/1) of the aid. In case of death, date of death was recorded and in turn calculated the survival.

We used Spearmańs Rho to establish the correlation between the progression rates of ALS with the external aids score. To establish the correlation with survival, we used the Kaplan-meier method, taking into account the classification described in the literature, where a progression rate <0.5=0; 0.5-1=1; and >1=2(1). A p value of <0.05 was considered significant.

Results: In total there were 144 patients. The progression rate was <0.5 in 46.4%; 0.5-1 in 27.5%; and >1 in 26.1%. In 73 patients (50.7%; mean age 57.4 years; SD 12.7) the need for external aids was recorded: 8.7% did not require aids; 24.6% required one aid; 10.1% required two aids; 13% required three aids; 23.2% required four aids; 11.6% required five aids; and 8.7% required six aids. We found a correlation between the progression rate and external aids score with Rho 0.27; $p = 0.01$.

In 71 patients (49.3%; mean age 61.8 years; SD 11.6) dates of death was recorded. In Kaplan-meier, a higher

proportion of deaths was found in patients with progression rates >1 (median survival IC 95%17.3-28.1 months) compared with progression rates between 0.5-1 (median survival 95%IC 32.4-56.3 months) and <0.5 (median survival 95%CI 67.7-124.9 months), p<0.00001.

Conclusion: From the initial ALS assessment we can have a prognostic factor of the need for external aids and survival using the progression rate of the disease.

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P26 MEDICATION EXPOSURE AND SURVIVAL IN ALS: AN OBSERVATIONAL STUDY USING PROPENSITY SCORE MATCHING

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Keywords: survival; propensity score matching; treatment

Background: There is little information on the effect of commonly used medications on progression and survival in ALS.

Objective: Examine the effect of exposure to commonly used medications on survival in a large cohort of ALS patients using propensity score matching.

Methods: An institutional database of self-reported measures and discrete health record elements (Knowledge Program Data Registry) was queried for the diagnosis of ALS, demographic information, self-reported measures (including ALSFRS-R), and medication use. Dates of death, when available, were added. Multiple imputation was used to obtain 50 complete datasets, and propensity score matching was performed on each imputed dataset to obtain treated and control groups better balanced for prognostically important covariates. Pooled hazard ratio estimates were used to examine the effect of medication exposure on survival. Seven a priori hypotheses (treatments) were examined.

Results: Of 1145 patients seen over 8 years, dates of death were known in 398. Median survival was 776 days (2.1 years). Medications and numbers of patients exposed included: riluzole (684); any antidepressant (597); aspirin (450); non-tricyclic antidepressant (443); statin (339); tricyclic antidepressant (274); and dextromethorphan-quinidine (127). The only treatment with a Bonferroni-corrected significant beneficial effect on survival was dextromethorphan-quinidine (HR 0.47, 95% CI 0.26-0.84, single-tailed p = 0.006), confirming findings from raw data. Non-significant favorable trends were seen with aspirin, non-tricyclic antidepressant, and statin. Surprisingly, a non-significant deleterious effect was found with exposure to riluzole. Similar results were obtained using alternative imputation/matching methods. Potential confounders and interactions were examined.

Discussion and conclusions: Short of a randomized controlled trial, matching algorithms on observational data presented here provide the best information on the causal effect of treatment. Although there may be several nonpharmacologic reasons for the apparent survival benefit of dextromethorphan-quinidine in ALS in this cohort, our findings warrant careful examination of this medication as a potential disease-modifying therapy.

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P27 MULTI-STEP ANALYSIS SHOWS THAT LATE ONSET ALS WITH SLOW PROGRESSION MAY REPRESENT A DISTINCT AETIOLOGICAL SUBGROUP

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Keywords: multi-step model, survival, ALS subgroup

Background: A multi-step model applied to populations with ALS shows a linear relationship between log incidence and log age of onset of ALS with a slope estimate of 5, consistent with a hypothesis that ALS is on average a six step process(1). However, there is considerable heterogeneity in survival rates of ALS(2), suggesting that stratification might reveal subgroups in which a different number of steps is needed to cause ALS.

Objectives: Use the multi-step model to test the hypothesis that ALS with short survival may represent a different entity from ALS with long survival.

Methods: Patients from five population registers in England, Ireland, Italy, Netherlands and Scotland were categorised as having short or long survival based on median disease duration from Kaplan-Meier analysis. Linear least squares regression was used to regress log incidence against log age of onset in the subgroups in each

register, which were then combined in a meta-analysis to derive the gradient of the best fit regression line. The slope estimate was taken as one less than the number of steps needed for disease onset.

Results: There was a linear relationship between log incidence and log age of ALS onset in both long (slope estimate of 3.6 (r^2 0.98, 95% CI 3.2-4.1)) and short (slope estimate of 5.7 (r^2 0.98, 95% CI 5.0-6.4)) survival groups. Subdividing the survival groups into young and old age of onset, using the arbitrary cut off of 50 years old, showed the old age of onset group with long survival had a very different slope estimate of 2.3 (95% CI 1.7-2.9) compared with the other three subgroups (old onset, short survival gradient 5.7 (95% CI 5.4-6.0); young onset long survival gradient 4.2 (95% CI 3.6-4.8) and young onset short survival gradient 4.6 (95% CI 4.0-5.2). Site of onset made no difference to slope estimate (bulbar onset, long survival gradient 4.0 (95% CI 3.7-4.4); spinal onset long survival gradient 3.4 (95% CI 3.2-3.7); bulbar onset, short survival gradient 5.5 (95% CI 5.0-6.0); spinal onset short survival 5.1 (95% CI 4.8-5.4).

Discussion and conclusions: We have shown that there is a distinct ALS subgroup comprising older people with slowly progressive disease in whom only three steps are required to trigger disease. The underlying aetiology must differ in at least one step between this subgroup and other forms of ALS and because there are only three steps, they may be more readily identified. This separate ALS subgroup may also respond differently to treatment and be relevant for clinical trial stratification.

Reference

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P28 CLINICAL CHARACTERISTICS OF ELDERLY PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: epidemiology, diagnostic delay, elderly

Background: Epidemiological studies of amyotrophic lateral sclerosis (ALS) show an increase in incidence with age up to the seventh or eighth decade, and a still unexplained steep decrease thereafter. Elderly patients tend to be more difficult to diagnose and to have a poorer prognosis, but evidence-based data for very old patients are lacking. Understanding the epidemiology and the course of the disease in this patient group is essential for early diagnosis, as well as for developing clinical interventions to improve quality of life and increase life expectancy among affected patients.

Methods: We analyzed retrospectively clinical data files from all ALS patients seen in our clinic during the last 15 years. Following data were recorded: age and form at onset; gender; diagnostic delay after first symptom; and familial history of ALS. Survival was known in all. Patients were divided into three groups by age at onset: lowest 10 percentile; highest 10 percentile; and a large middle group.

Results: The patient database used included 984 patients: 99 were included in the young-age group (43 years and below); 96 patients were in the old-age group (76 years and above); and 789 patients constituted the middle group. Among patients with an old age at onset there was a significantly higher proportion of patients with bulbar presentation ($p<0.015$) and female gender ($p<0.008$). Survival was significantly lower ($p<0.0001$) among the elderly patient group as compared to the other patients. Familial case histories were extremely rare in the old age group ($p<0.000$). Surprisingly, the diagnostic delay was slightly shorter in the elderly patient group as compared to younger patients, probably as a consequence of the faster progression, but this difference was not statistically significant.

Conclusions: ALS has some specific clinical characteristics in elderly patients: a higher proportion of women; bulbar cases; and poorer survival. These characteristics have to be recognized in order to improve diagnosis, prognosis and quality of life among patients in this age group.

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P29 HEMATOLOGICAL PROGNOSTIC MARKERS OF SURVIVAL IN DUTCH AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Keywords: prognostic blood biomarkers, survival

Background: In search of biomarkers for amyotrophic lateral sclerosis (ALS), hematologic laboratory tests have been proposed as prognostic markers for survival. However, current evidence is sparse (1). Since laboratory tests are easily accessible, they might provide a simple way to monitor disease in clinical trials, and might improve our insight into the pathogenesis of ALS, an important step for developing therapeutic interventions.

Objectives: To investigate which laboratory tests might be of prognostic value for survival in ALS.

Methods: In a retrospective design, data were collected from patients referred to the outpatient clinic for motor neuron diseases between January 2006 and December

2012. For diagnostic purposes, blood samples had been obtained for all of these patients. Blood samples of patients diagnosed with definite, probable, as well as probable-laboratory supported, and possible ALS were included. Laboratory tests that were present for at least 40% of the patients were considered for analysis, these included: estradio; estrone; testosterone; unbound testosterone; sex-hormone binding globulin; phosphate; aspartate transaminase (AST); alanine transaminase (ALT); gamma-glutamyl transpeptidase, albumin; creatine kinase (CK); C-reactive protein, cell counts of leukocytes; lymphocytes; monocytes; neutrophils; basophils; eosinophils and thrombocytes; haemoglobin; hematocrit; mean corpuscular volume, mean corpuscular haemoglobin; mean corpuscular hemoglobin concentration; thyroid-stimulating hormone (TSH); vitamin E; Borrelia IgG titre; and Borrelia IgM titre.

In total, 28 blood tests were analyzed and their association with survival was assessed. Multivariate Cox models handling the laboratory tests as continuous variables were used to control for confounding variables (gender, age at onset, site of onset, *C9orf72* repeat expansion). Date of death or date of censoring was used as an endpoint. False discovery rate (Benjamini-Hochberg) was used to control for multiple testing.

Results: 797 patients were included. Clinical characteristics were comparable with our Dutch population-based cohort described elsewhere: 58.6% male;, mean age at onset 63.3 years (SD 11.7); 33.5% bulbar onset; and 6.4% with a *C9orf72* repeat expansion. Multivariate analyses revealed that none of the 28 laboratory tests were significantly associated with survival, neither when samples of a subset of patients with definite and probable ALS were selected for analysis (all $p>0.05$).

Discussion and conclusions: In this study, we found no new hematological markers of prognostic value, nor could we confirm albumin as prognostic biomarker as was found earlier. Moreover, cell counts of leukocytes, lymphocytes and monocytes, CK, liver enzymes, and TSH were not associated with survival, as reported earlier. Some of the blood tests were studied for the first time in relation to ALS survival. Since literature is sparse and sometimes conflicting, further replication of these findings in other cohorts is needed.

Reference

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P30 CREATINE KINASE ENZYME LEVEL CORRELATES POSITIVELY WITH THE SERUM CREATININE AND LEAN BODY MASS, AND IS A PROGNOSTIC FACTOR FOR SURVIVAL IN ALS

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Keywords: creatine kinase, creatinine, biomarker

Background: Creatine kinase (CK) enzyme is commonly elevated in patients with ALS. CK catalyses the reversible conversion of phosphocreatine, an energy reservoir in muscle and nerve cells, to creatine generating ATP. Increased athletic performance and neuroprotective effects have been attributed to the administration of creatine and creatine analogs. As CK is required for creatine metabolism, the genes for the various CK isoenzymes are up-regulated in individuals involved in activities that require muscular and cardiorespiratory endurance. In addition, serum creatinine, which is the by-product of the reaction catalysed by creatine kinase, has been suggested as a muscle mass biomarker in ALS, related to the rate of disease progression. These facts suggest that CK may be up-regulated in ALS to combat metabolic stress and hence may influence prognosis.

Objectives: To explore the potential of CK as a prognostic biomarker in ALS.

Method: This is an observational cohort study, using the clinical database from the Olesoxime (TRO19622) investigational medicinal product trial. This trial involving 512 patients with ALS was conducted across 15 European centres (2009-2011). The patients were followed up at 3 monthly intervals for 18 months, with monitoring of various biochemical and haematological parameters, including CK. The relationship between the longitudinal CK data and overall survival was assessed using time dependent Cox regression analysis.

Results: The baseline CK was raised in 52% of the participants. The mean CK for the whole cohort was 257 U/L, with the maximum recorded value of 1843 U/L (male normal: 24 - 195 U/L, female normal: 24 - 170 U/L). The median CK was 190 U/L. 90% of the patients had CK less than 500 U/L and only 6 patients had a level above 1000 U/L. The mean CK was significantly higher in males then in females and was higher in the participants with limb onset disease compared to bulbar onset disease. There was a strongly positive relationship between CK and the estimated lean body mass of the affected individuals throughout the follow-up period. CK levels correlated positively with the serum creatinine levels. However, the CK levels did not co-relate strongly with the manual muscle scores at any time point. The Cox proportional hazard model suggested that the CK was an independent factor for survival; one unit increase in logCK results in an individual being 0.74 times as likely to die at any given time point ($p = 0.01$).

Conclusions: This study highlights the potential usefulness of CK as a prognostic marker in ALS, most likely through its crucial role in energy metabolism.

Acknowledgement: The steering committee of TROPHOS (the Biotechnology company developing Olesoxime) kindly provided the database from the Olesoxime trial to conduct this study.

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P31 VITAMIN D: NOT PROTECTIVE IN ALS

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Keywords: vitamin D, neuroprotection, prognosis

Background: Vitamin D deficiency has been associated with poorer prognosis in ALS. Better understanding of the role of vitamin D in ALS is needed to determine whether trials of systematic supplementation are justified.

Objectives: Our aim was to report vitamin D levels during the course of ALS and to evaluate its relationship with clinical as well as biological parameters at diagnosis and with disease progression.

Methods: We prospectively collected vitamin D serum concentrations from 125 consecutive ALS patients. Cox proportional hazard models analyzed the relationship between vitamin D concentrations, clinical parameters and survival.

Results: The mean vitamin D concentration was below our laboratorys lower limit of normal ($p<0.0001$), and did not change during the course of the disease. The concentrations were higher in patients with bulbar-onset ($p = 0.003$) and were negatively associated with Body Mass Index ($p = 0.0095$). Models with ALSFRS-R (ALS Functional Rating Scale-Revised) and BMI (Body Mass Index) as covariates showed that vitamin D concentrations predicted worse prognosis.

Conclusion: The distribution of vitamin D concentrations in our cohort was consistent with previous reports. Surprisingly, we noted a negative effect of higher vitamin D levels on prognosis in ALS. More detailed research is warranted to determine whether manipulation of vitamin D could be beneficial to patients.

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P32 PITFALLS IN THE USE OF ALSFRS SLOPE IN THERAPEUTIC TRIALS, AND ITS VARIABLE RELATIONSHIP TO SYMPTOM ONSET

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Keywords: progression, ALSFRS, prognosis

Background: The rate of accumulation of disability varies significantly between individuals with ALS. The current standard for measurement is the ALS Functional Rating Score (ALSFRS). The rate of ALSFRS decline is frequently anchored to reported symptom onset, typically defined as the earliest perception of motor deficit.

Objectives: To assess whether ALSFRS consistently reflects disease progression over time, specifically contrasting the time interval from symptom onset to therapeutic trial enrolment against repeated measurements within the trial assessment period.

Methods: We analysed data from 18 pooled negative therapeutic trials (using the PRO-ACT database and LiCALS data), describing the spread of ALSFRS scores at enrolment and the progression across intervals in time (mean 8.8 data points over 11.1 months). We contrasted ALSFRS rate calculation with and without incorporation of subjective symptom onset. We also compared the reported symptom onset with a temporal estimate of maximum ALSFRS score (ie no disability) using a back-extrapolation of the ALSFRS slope.

Results: Disability accumulation during these negative clinical trials was typically faster than anticipated, relative to a prior progression rate estimated from the patients reported symptom onset. The median window from reported symptom onset until disability was severe enough to register on the ALSFRS was 5.8 months (interquartile range 14.2). This disparity was lower in patients with more rapidly-progressive disease, and also if trial enrolment took place further into their disease course.

Discussion and conclusion: The findings suggest that short duration therapeutic trials may not be robust if judged by impact on ALSFRS decline. There is an inherent conflict between the desire to enrol participants early in disease versus the reduced reliability of symptom onset-defined progression rates. This stratification inaccuracy is mitigated in more rapidly-progressing cases. Overall the findings reinforce the need for an objective pharmacodynamic biomarker.

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P33 VALUES: THE EFFECTS OF LOW ESTROGEN ON EXECUTIVE FUNCTIONING IN A NATIONAL SAMPLE OF ALSCI INDIVIDUALS SUGGESTS A NEUROENDOCRINE MODEL OF DISEASE ONSET

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Keywords: estrogen, neuroprotective factor, cognition

Background: Amyotrophic lateral sclerosis (ALS) is characterized by behavioral and cognitive deficits, consistent with frontotemporal lobar degeneration (FTLD) while in the absence of dementia, in up to 50% of affected individuals (ALSbi and ALSci). Recently, the neuroprotective benefits of estrogen have been posited in several neurodegenerative disorders, particularly in the early postmenopausal period.

Objectives: To explore gender differences and evidence the role of estrogen as a potential neuroprotective factor in executive functioning decline in a non-demented ALSci female sample.

Methods: We evaluated 73 female and 69 male ALSci patients aged 28-75 from 10 multidisciplinary ALS clinics to explore the potential of estrogen as a neuroprotective factor. Participants exhibited 2 or more deficiencies on the Penn State Brief Exam of Frontal and Temporal Dysfunction Syndromes (PSFTS), a validated set of measures to identify cognitive and behavioral impairment indicative of emerging FTLD. We examined cognitive executive functioning findings and Frontal Behavioral Inventory (FBI) profiles, assessing behavioral impairment for ALSci males and females, by non-bulbar and bulbar onset. Female ALSci participants were further classified as having high or low levels of estrogen, based upon age and medical chart review.

Results: Mann Whitney U analyses indicated bulbar onset females performed significantly worse on letter fluency (LF) than those with limb-onset, with significant differences in LF T-scores ($p = .003$), and 'F' ($p = .003$), 'A' ($p = .003$), and 'S' ($p = .000$) raw scores. For the low estrogen bulbar subgroup, LF findings were significantly worse than for the high estrogen bulbar subgroup (T scores ($p = 0.012$), 'F' ($p = 0.008$), 'A' ($p = 0.02$), 'S' ($p = 0.005$) raw scores). Conversely, LF findings for high estrogen limb and bulbar onset were statistically equivalent. Consistent with the high estrogen females, bulbar- ($N = 30$) and limb- ($N = 43$) onset males were equivalent in LF T-scores, as well as F, A, S Raw Scores ($p > 0.05$). 59 ALSci females and 69 ALSci males who also displayed 2 or more findings on the FBI represented the ALSbi samples. Bulbar-onset females scored significantly higher on the trait of apathy than limb-onset ($\chi 2=4.50$; $p = 0.034$), while no FBI differences were evident between male bulbar- and limb-onset.

Conclusion: This study evidences the potential for estrogen to mitigate the executive functioning declines often associated with ALS. Glial cells are affected by estrogenic compounds, regulating remyelination, edema formation, extracellular glutamate levels and the inflammatory response after brain insult. Additionally, estradiol induces the expression and release of growth factors by glial cells that promote neuronal survival. Findings of cognitive and behavioral differences between bulbar- and non-bulbar onset groups were both female specific and estrogen driven, supporting the theory of a neuroprotective benefit of estrogen in ALSci females, particularly for a bulbar associated model of 'bottom-up' disease onset.

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P34 STAGING COMMUNICATION ABILITY IN ADVANCED ALS PATIENTS

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Keywords: communication, tracheostomy, invasive ventilation

Background: As our ability to provide care and treatment improves, patients with ALS are living longer. We are seeing changes in cognition and ability to control eye movements, especially in individuals who require invasive ventilator support. Some advanced patients progress to the point where they become locked in or without any way to communicate.

Objectives: To define a disease trajectory pattern in ventilator dependent ALS patients that might predict development of a locked-in state.

Methods: Retro-prospective review of disease progression of 82 patients (31 female; 51 male) seen at our CMC ALS center who became ventilator dependent grouped according to communication ability at most recent visit or death using clinical ALS communication stages (Hayashi, 2013).

Results: At death or most recent clinic visit, 45 patients were in Stage I (able to communicate in sentences); 27 patients were in Stage II, III or IV (difficulty with communication); and 10 patients were in Stage V (unable to communicate by any means). We identified age at onset (median=53 years, 95% CI=51-55) and site of onset (lower limb n=29; upper limb n=28; bulbar $n = 22$; respiratory $n = 2$). Median length of time from symptom onset to diagnosis was 10 months (95% CI=9-12 months). Overall median length of time from onset to death was 63 months (95% CI=51-75 months). The median length of time from onset to tracheostomy with invasive ventilation (TIV) for the 3 stages was found to be statistically significantly different ($p = 0.0299$): Stage I (38

months; 95% CI=28-53); Stage II, III, IV combined (28 months; 95% CI=25-36); Stage V (29 months; 95% CI=18-40), as was the median time from diagnosis to TIV for Stage I (20 months; 95% CI=13-34); Stage II-IV (20 months; 95% CI=13-23), and Stage V (12 months;95% CI=7-23) ($p = 0.0330$).

Discussion and conclusions: Our observations confirm that there is no significant difference in length of time from onset to death in ALS communication Stages I-V, but that there is a statistically significant difference in length of time from onset to TIV in Stage I. Patients in Stage I who can communicate in full sentences (33% via speech/mouthing; 31% via eye-gaze; 20% via writing/typing; 16% via scanning) have a longer time from onset to TIV. In addition, we have shown that length of time from diagnosis to TIV is significantly different in Stage I vs. II-IV vs. V. Ongoing analysis will relate measures of cognition to oculomotor assessment and optokinetic nystagmus to more clearly identify characteristics of each communication stage.

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P35 CLINICAL ASSESSMENT PROTOCOL OF THE OROFACIAL MUSCULATURE, WITH GRAVITY AND FATIGUE MARKERS, TARGETED TO PATIENTS WITH MOTOR NEURON DISEASE

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Keywords: stomatognathic system, assessment, fatigue

Background: Speech therapy assessment aims to map the structures of the stomatognathic system, identifying the points that can be triggers of functional changes, such as dysphonia, dysarthria and dysphagia. This detail is crucial, considering that functional alterations can be indicators of severity or even markers of evolution of the disease, especially when it comes to an evolutionary disease such as motor neuron disease.

Objective: To present a clinical assessment protocol of the musculature of the stomatognathic system, with gravity and fatigue markers, targeted to patients with motor neuron disease.

Methods: A speech evaluation protocol of the stomatognathic system muscles was drafted, with gravity and fatigue indicators; for the following muscle groups: lips; tongue; cheek; masticator and soft palate.

Results: For each muscle group, records of the assessment were defined, each of these records has five categories, with well defined clinical criteria based on symmetry, posture, aspect, range of motion and ability to perform resisted movement (against resistance). Each of these items can be assessed 1 to 5 where: 1 is equivalent to normal; 2 to mild; 3 to moderate; 4 to severe; and 5 to serious inability of execution. In addition, the protocol evaluated the number of times each contracting muscle participated in isotonic movement, before presenting the first sign of fatigue, which can occur by reducing the range of motion, speed or accuracy or even the need to recruit other muscle groups for the finalization of the proposed task. The protocol can complement the assessment of swallowing, breathing, voice and speech; since changes of orofacial structures can lead to changes in the stomatognathic functions.

Discussion and conclusions: The registration of graduate clinical occurrences are potential indicators of severity and can be re-applied over time, in conjunction with the functional scales, to better assess the evolution of the disease. From a therapeutic point of view, learning more about each aspect of the orofacial musculature can help detail the professional in the best indication of the type of exercise, as well as in the definition of the series of exercises to be prescribed. The best indication of exercise so far, has been for the patient to carry out isotonic activity and stop before the slightest sign of fatigue (muscle or respiratory fatigue). To this end, it is necessary that the therapist establishes this reference of fatigue, from the (re) assessment clinic.

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Theme 3 Biomarkers and Outcome Measures

P36 PANEL OF OXIDATIVE STRESS AND INFLAMMATION MARKERS IN SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: oxidative stress, inflammation, biomarkers

Background: Irrespective of the cause, downstream pathophysiological events that contribute to neurodegeneration in ALS include oxidative stress and inflammation. Oxidative stress and inflammation biomarkers have been repeatedly and independently found in ALS but a lot of discrepancies related to these findings have been reported. Although this phenomenon is non ALS-specific, the exploration of these mechanisms may improve the knowledge of ALS pathogenesis. We conducted a preliminary study to explore some relevant markers of oxidative stress and inflammation and to discuss their involvement and their link in ALS, independently from other parameters linked to oxidative stress such as vitamins, metals and homocysteine.

Methods: We enrolled 10 ALS patients and 10 sex and age-matched controls without any neurological disorders. We measured the activities of GPx (glutathione peroxidase), GR (glutathione reductase), SOD (Super Oxide Dismutase). Serum Total Antioxydant Status (TAS), malondialdehyde (MDA), 8OH-DG (8-Oxo-2′-deoxyguanosine), GSH (glutathione) and GSSG levels were measured. Moreover, we analysed concentrations of some cytokines (IL-10, IL-6, IL-8, TNFa). We also measured the concentrations of 5 vitamins (serum and erythrocyte B9, B12 and A, E and C), homocysteine and the following metals: Cu, Zn, Mn, Se and Fe. We compared the levels of these markers between both populations and we analysed the relationships between biological parameters and clinical characteristics and between biological parameters together.

Results: There was a significant decrease in TAS levels in ALS patients compared to controls ($p = 0.027$) and elevated concentrations of 8OH-DG and MDA in ALS patients ($p = 0.014$ and 0.011, respectively). Status of glutathione was also altered in ALS patients with an increase in GSSG ($p = 0.034$) and a decrease in GSH ($p = 0.034$), leading to a significant higher GSSG/GSH ratio ($p = 0.022$).

We noted an increase in the following inflammation markers in ALS patients: IL-6 ($p = 0.0079$) and IL-8 ($p = 0.009$). We also observed a trend to an increased concentration of TNFa in ALS patients ($p = 0.06$). We found an inverse relation between homocysteine concentrations and ALSFRS at diagnosis ($p = 0.02$) and a positive correlation between Cu levels and ALSFRS decline ($p = 0.0037$). We observed a positive correlation between IL-6 concentrations and the ratio GSSG/GSH ($p = 0.045$) and an inverse correlation between IL-6 concentrations and SOD activity ($p = 0.017$).

Discussion: We confirmed the alteration of redox status as well as inflammation mechanism in ALS patients and we observed a link with some clinical parameters. This study is a proof of concept that the exploration of all these markers of oxidative stress could be feasible and independently from vitamin and metals levels. All these parameters have to be included in prognosis studies. These promising results encourage us to pursue this study with collection of combined inflammation and oxidative stress markers, confusion factors and clinical data to rigorously evaluate these relationships.

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P37 ASSESSMENT OF A MULTIPLE BIOMARKER PANEL AND ITS COMBINED USE FOR AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: pNfH, CHIT, cystatin C

Background: To date there are no validated biomarkers for amyotrophic lateral sclerosis (ALS). The aim of our study is to verify the most promising biomarker candidate in a multiple biomarker panel and to assess whether the combined use of different biomarkers could improve diagnosis in ALS.

Methods: 40 patients with definite sporadic ALS (SALS) and 40 with other neurological diseases were evaluated. Cerebrospinal fluid (CSF) pNfH, S100-β, cystatin C, and CHIT levels were assayed by two-site solid-phase sandwich ELISA. The neurological status of SALS patients was assessed by the revised ALS Functional Rating Scale (ALSFRS-R), the worsening of neurological function was determined by monthly decline of ALSFRS-R, and the

progression of the disease was evaluated using the progression rate.

Results: CSF pNfH and CHIT levels were higher and CSF cystatin C levels were lower in SALS cases than in controls. Multivariate logistic regression confirmed the association between the presence of ALS and the levels CSF pNfH, CHIT and cystatin C (age and sex adjusted). In ALS, CSF pNfH positively correlated with the progression rate, and higher CSF pNfH levels were associated with faster rate of decline in the ALSFRS-R. Using receiver operating curve (ROC) analysis, an optimal CSF pNfH cut-off value of 437 ng/l discriminated SALS cases from neurological subjects, with a sensitivity of 97.3% and a specificity of 83.8%. Using a cut-off for CSF CHIT at > 1593.779 ng/l gave a sensitivity of 83.8% and a specificity of 81.1%. The combined use of pNfH and CHIT as biomarkers of ALS obtained a sensitivity of 83.8% and a specificity of 91.9%.

Conclusions: CSF pNfH may be the most promising biomarker candidate for ALS, and the combined use of CSF pNfH and CHIT as biomarker of ALS may improve the diagnostic accuracy in the clinical work-up.

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P38 DECREASED LEVEL OF SERUM AUTOANTIBODY AGAINST G72 IS A BIOSIGNATURE OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: G72, biomarker, autoantibody

Background: G72 is a well-known genetic marker in mental disorders including schizophrenia, major depression and bipolar disorders. In G72 transgenic mice and G72-transfected U87 glioma cells, exogenous G72 increases the level of mitochondrial reactive oxygen species and causes mitochondrial dysfunction. The former two pathological phenomena are related to neurodegenerative diseases such as AD, PD and ALS. Since the proved evidence of G72-induced oxidative stress and mitochondrial dysfunction has been implicated, the level of G72 or G72-related molecules might therefore be associated with ALS.

Objectives: We aimed to determine whether the serum level of an autoantibody against G72 has potential as a biomarker for the diagnosis and clinical evaluation of patients with ALS.

Methods: Serum autoantibody against G72 was measured using an enzyme-linked immunosorbent assay designed in-house in 82 patients with ALS, 45 patients with AD, 43 patients with PD, and 88 healthy adults. The level of autoantibody against G72 was compared and statistically correlated with gender, age at onset, duration, and ALSFRS-R. Finally the ROC curve was employed to determine the performance of autoantibody against G72 as a biomarker for diagnosis of ALS.

Results: The mean concentration of serum autoantibody against G72 in patients with ALS was 0.09 μg/ml. This was significantly lower than the concentration in normal controls (0.40 μg/ml), patients with AD (0.49 μg/ml), and patients with PD (0.58 μg/ml). There was no correlation between the level of the autoantibody and clinical characteristics of ALS, including gender, age at onset, disease duration, and ALSFRS-R. ROC curve was performed and the autoantibody against G72 exhibited 95.00% accuracy, 91.46% sensitivity, and 92.05% specificity for diagnosis of ALS when the cut-off value was 0.167 μg/ml. The AUC was 0.9627, indicating that serum level of autoantibody against G72 may be useful as a biomarker for ALS.

Discussion: We found that serum autoantibody against G72 was lower in all subgroups of patients with ALS when they were classified by gender, type of onset, age, duration, and disease severity. The phenomenon of low level of autoantibody against G72 was present even in the very early stage of ALS after clinical symptom onset and regardless of whether the disease progression was minor or severe. This suggests that the low level of autoantibody against G72 is a relatively early indicator of ALS.

Conclusions: To our knowledge, this is the first study to elucidate the relationship between G72 and ALS. The autoantibody against G72 was significantly decreased in the serum of patients with ALS, and this phenomenon was present even at the very early stages of symptom onset. Our results suggest that G72 might be involved in the pathogenesis of ALS.

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P39 PROTEOMIC SEARCH FOR EARLY BIOMARKERS FOR AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: cerebrospinal fluid, biomarkers, proteomic search

Background: Until now, the diagnosis of Amyotrophic lateral sclerosis (ALS) is based on the combination of clinical and neurophysiological observations. Specific biomarkers are urgently required for early diagnosis of ALS with high sensitivity and specificity, and for the monitoring and evaluation of the efficacy of new treatments. Also, little information is available from patient material on the pathological chain of events which may uncover the core mechanism of motor neuron death.

Objectives: A comprehensive proteomic search strategy is applied to the search for promising specific biomarkers in cerebrospinal fluid (CSF) of patients with ALS. CSF was acquired applying strict standard operation procedures from three groups of patients: R: rapidly progressive ALS; S: slowly progressive ALS; and C: controls without ALS. The proteomes of all groups were analysed and compared to identify specific biomarker candidates discriminating these 3 groups.

Methods: To achieve sufficient protein amounts, pooled CSF samples of each group were used. Equal volumes of CSF from 11, 6, and 9 patients were combined from R, S and C, respectively. CSF-pools were concentrated by ultrafiltration and fractionated using a 2-dimensional chromatographic method (1D-size exclusion chromatography followed by semiautomatic parallelized 2D-anion exchange chromatography[1]). For each pooled sample we got 1560 2D-fractions. Fractions with protein conc. >0.03 mg/ml (338 fractions per pool) were selected, digested by Trypsin + LysC and analysed by LC-MS/MS in technical triplicates. Data were processed by Proteome Discoverer and Sieve 2.0 (Thermo Scientific) and filtered using Excel™ and the Biomarker Analyser (X-CASE).

Results: In all analysed CSF samples we identified 1824 protein groups (5185 single proteins) supported by 1 peptide, 676 (3494 single proteins) with 2 peptides. Control CSF vs. CSF of patients with slowly and rapidly progressing ALS showed significantly increased or decreased proteins for each subgroup, some of which are strongly associated with brain tissue and neuronal death. The analysis is still ongoing.

Conclusions: With the identification of significantly increased or decreased protein fractions in the CSF of slow and rapidly progressing ALS patients we will be able to extract determinants of disease course. These hits will serve as a starting point for detailed analyses of pathways which may serve as prognostic indicators and in the development of treatment targets. Combined with genomic data, this level of proteomic profiling will enable the meta-analysis of big data previously not available in ALS.

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P40 AN EXHAUSTIVE PROTEOMIC ANALYSIS OF THE CEREBROSPINAL FLUID OF AMYOTROPHIC LATERAL SCLEROSIS PATIENTS, THE SEARCH FOR POTENTIAL NEW BIOMARKERS

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Keywords: biomarkers, proteomics, CSF

Background: The early diagnosis, disease monitoring and treatments that could mitigate disease progression in amyotrophic lateral sclerosis (ALS) will depend on the as yet impossible task to measure objectively the molecular changes underlying the development of this disease. The simultaneous analysis of different tissues and biological fluids using powerful and revolutionary proteomic techniques has allowed us to define molecular changes that will lead us to a better understanding of ALS.

Objectives: Performing an exhaustive description of the CSF proteome composition in ALS patients compared to non-ALS controls that will enable us to report changes at peptide, protein and biological pathways level.

Methods: The CSF proteome was analysed using a novel proteomics workflow, TMT 10 calibrator developed by Proteome Sciences. Here a disease-relevant tissue is used as a source of reference proteins which is spiked into the relevant fluid samples at a relatively high concentration, ensuring that the majority of mass spectrometry analysis is performed on peptides found in the tissue that are also seen in the fluid. By using the isobaric Tandem Mass Tag technology it is possible to make a multi-point calibration curve from the reference tissue against which the fluid levels of the same peptide can be calculated. In the present study 3 ALS samples and 3 non ALS controls have been labelled and spiked with 4 different concentrations of ALS brain pool lysate, to be later compared. This technique has been used in combination with the Sysquant-phospho enrichment and fractionation workflow to process these samples in order to increase the number of peptides and phospho peptide identified.

Results: 57.861 peptides from the test samples have been identified of which 1587 were significantly regulated between the ALS and non-ALS groups. At protein level, 13.423 proteins have been detected of which 322 were significantly regulated between the two groups.

Discussion and conclusions: In a hypothesis-driven data analysis, based on the search proteins that have been

previously proposed as biomarkers for ALS (1-2), a significant regulation at peptide level was detected for Internexin, TDP43, Neurofilament heavy, medium and light polypeptides, RNA binding protein FUS and Dynactin subunit 1. In addition, we performed a data driven analysis; reporting the top 10 up and top 10 down regulated proteins and peptides in ALS as well as the most enriched molecular functions and KEGG pathways for the regulated peptides.

We have defined a biological framework of the disease and the vast amount of data generated here will be an important source of information for the scientific community working on a better understanding of ALS.

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P41 THE EXTRACELLULAR DOMAIN OF P75 NEUROTROPHIN RECEPTOR IS PRESENT IN BOTH URINE AND CEREBROSPINAL FLUID OF PEOPLE LIVING WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: CSF, urine, biofluid biomarker

Background: The neurotrophin receptor p75 (p75NTR) is re-expressed on motor neurons in ALS[1] and in healthy motor neurons cultured with mutant SOD1 glia[2]. It was previously reported that p75 can mediate apoptosis when expressed in adult neurons and its extracellular domain ($p75NTR^{ECD}$) shed from diseased neurons[3]. We have previously shown that $p75NTR^{ECD}$ is significantly increased in the urine of ALS patients compared to healthy controls and people with Multiple Sclerosis and Parkinsons disease[4]. It is not known if urinary $p75NTR^{ECD}$ in ALS patients reflects shedding of $p75NTR^{ECD}$ from central and spinal motor neurons, or from the periphery.

Objectives: We wish to determine if $p75NTR^{ECD}$ is elevated in the cerebrospinal fluid (CSF) of ALS patients compared to other neurological controls and confirm our urinary findings. Data from this study will give some indication of the specificity of the marker and source of urinary $p75NTR^{ECD}$.

Methods: CSF and urine were sampled from ALS and control patients from the United Kingdom (Sheffield), Australia (MND clinic South Australia) and the USA (Miami Clinic). $p75NTR^{ECD}$ levels were assayed using a specific ELISA, with CSF dilution normalised with total protein content and urine with creatinine. CSF was sampled from 13 ALS patients (age 5911 years). Control CSF was from people with headache, peripheral neuropathy, paraparesis and chronic inflammatory demyelinating polyneuropathy (age 5912 years $n = 9$). Urine samples were from 53 sporadic ALS patients (age 67.711.8 years, 17 bulbar-onset, 27 limb onset) and 23 healthy individuals (age 5112.9 years).

Results: CSF levels of $p75NTR^{ECD}$ were significantly elevated in ALS patients with bulbar ($p = 0.02$) and upper limb ($p = 0.01$) onset compared to neurological controls by about twofold. Consistently, urinary $p75NTR^{ECD}$ levels in limb and bulbar onset were also significantly elevated ($p = 0.0001$) compared to healthy controls.

Discussion and conclusion: $p75NTR^{ECD}$ appears elevated in both CSF and urine of ALS patients. This is further evidence for elevated biofluid $p75NTR^{ECD}$ as a biomarker of ALS. Future efforts are identifying the specificity and the accuracy of $p75NTR^{ECD}$ levels in biofluids as a prognostic and as a progression marker for ALS patients.

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P42 LONGITUDINAL CHANGES IN URINARY P75 NEUROTROPHIN RECEPTOR EXTRACELLULAR DOMAIN LEVELS AS A DISEASE PROGRESSION BIOMARKER IN ALS PATIENTS

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Keywords: biomarker of disease progression, longitudinal study, p75NTR

Background: Objective biomarkers that change with disease progression are needed for ALS clinical trials. Longitudinal studies are yet to confirm a marker that changes as disease progresses. We have previously shown that the p75 neurotrophin receptor extracellular domain ($p75NTR^{ECD}$) is significantly increased in the urine of ALS patients compared to healthy controls and people with Multiple Sclerosis and Parkinsons[1].

Objectives: We wish to determine if urinary $p75NTR^{ECD}$ changes longitudinally across individual ALS patients. We will also ask if it is possible to produce mathematical models of disease progression and onset using urinary $p75NTR^{ECD}$ levels.

Methods: Urinary $p75NTR^{ECD}$ levels were assayed longitudinally in 35 sporadic ALS patients using an ELISA. At least 2 consecutive measurements were taken every 3-5 months. First and last samples were collected a mean of 17.2 (range 4.4-72.7) and 27.2 (range 10.7-85.7) months after symptom onset. The average age was 67.111.6 years, with 15 females, 12 with bulbar and 23 with limb onset. All ALS patients were also enrolled in the Australian Motor Neuron Disease Registry (AMNDR) where progression was recorded using the revised ALS function rating scale (ALSFRS-r) at the time of urine collection. All samples were normalised for dilution using urinary creatinine concentrations.

Results: Urinary $p75NTR^{ECD}$ levels measured over an average of 10 months (range 2.7-37.0) significantly ($p<0.0001$) increased in ALS patients ($n=35$) over time. There was also a strong correlation ($r=-0.4438$, $p<0.0001$) between the increase in urinary $p75NTR^{ECD}$ and the decline in ALSFRS-r score. Mean ALSFRS-r scores were 40 (27-46) and 32 (14-45) at the time of first and last samples, respectively. Modelling was done using longitudinal $p75NTR^{ECD}$ levels. $p75NTR^{ECD}$ levels increased by an average of 0.2ng/mg/month across the 35 patients and levels of 2-5ng/mg creatinine was predicted at onset. There was no correlation between longitudinal urinary $p75NTR^{ECD}$ level changes and gender, age, or site of disease onset throughout disease progression in ALS patients.

Discussion and conclusion: Longitudinal data demonstrates the utility of $p75NTR^{ECD}$ as a disease progression biomarker. To date, urinary $p75NTR^{ECD}$ is the first biochemical marker that changes as disease progresses and therefore shows promise for use in future clinical trials of ALS treatments.

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P43 INCREASED TDP-43 IN THE SKIN OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: immunohistochemistry, skin, TDP-43

Background: Amyotrophic lateral sclerosis (ALS) is a devastating degenerative disease of upper and lower motor neurons with undetermined cause. The lack of bedsores even in the terminal stages in ALS patients is taken to be characteristic. Several studies of skin in ALS patients have shown unique morphological and biochemical alterations. After its initial identification in 2006, numerous studies have now confirmed that abnormal neuronal and glial inclusions composed of the TAR DNA-binding protein 43 (TDP-43) are the neuropathological hallmark lesions in several neurodegenerative disorders, which can be subsumed under the term TDP-43 proteinopathies. This includes sporadic frontotemporal lobar degeneration with tau-negative and ubiquitin-positive inclusions (FTLD-U) with and without motor neuron disease (MND), familial forms of FTLD-U with mutations in the progranulin gene (GRN), valosin-containing protein (VCP) and linkage to chromosome 9p, as well as most forms of ALS with the exception of familial ALS with SOD-1 mutations. In addition, concomitant TDP-43 pathology is present in a subset of other neurodegenerative diseases, such as Alzheimer's disease (AD), Lewy-body dementia (LBD) and parkinsonism-dementia complex of Guam. It is unknown, however, whether TDP-43 positive structure are present in skin of sporadic ALS (SALS).

Objective: To study TDP-43 in the skin of ALS patients.

Methods: Skin biopsy specimens were taken from the left biceps from 18 SALS patients (mean age 63.5 years) and from 15 controls with other neurodegenerative diseases (60.2 years). Routine formalin-fixed paraffin-embedded 6 μm sections were immunostained according to standard techniques. A densitometric analysis was performed using an image analysis system.

Results: The proportion of TDP-43-positive cells in the epidermis in SALS patients is significantly higher ($p<0.001$) than in controls. There was a significant positive relationship ($r=0.62$, $p<0.02$) between the proportion and duration of illness in SALS patients. The optical density of TDP-43-positive cells in the epidermis in SALS patients is markedly stronger ($p<0.001$) than in controls. There was a significant positive relation ($r=0.72$, $p<0.01$) between the immunoreactivity and duration of illness in SALS patients. Also, in TDP-43-positive cells in the epidermis of ALS patients the optical density showed a progressive increase in relation to the proportion. This positive correlation was highly significant ($r=0.75$, $p<0.01$).

Discussion and conclusions: The increased TDP-43 immunoreactivity of skin does not reflect nutritional status-or activity-dependent skin remodeling. From these considerations, our results as to the increase of TDP-43 immunoreactivity of skin in SALS patients might indicate

an augmentation of TDP-43 content due to reduced degradation, increased synthesis, and/or increased binding of circulating TDP-43 by skin components. These data suggest that changes in TDP-43 identified in the skin of SALS patients are likely to be related to the disease process and that metabolic alterations of TDP-43 may take place in the skin of patients with SALS.

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P44 METHODOLOGICAL COMPARISON OF EXTRACELLULAR VESICLE EXTRACTION AS A NOVEL SOURCE OF BIOMARKERS IN ALS

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Keywords: exosomes, biomarkers, cerebrospinal fluid

Background: There is currently a critical and unmet need for biomarkers sensitive to disease activity in ALS. Extracellular vesicles (EVs) have biologically important functions, including secretion of unwanted proteins and the delivery of signals to other cells (1). Extraction of EVs from the serum and cerebrospinal fluid (CSF) of ALS patients might enrich less abundant, more disease-specific biomarkers (2).

Objectives: To optimise extraction methodologies of EVs from the serum, CSF and induced pluripotent stem cell (iPSC)-derived neuronal cultures of ALS patients for subsequent characterisation.

Methods: EVs were purified from serum, CSF and iPSC motor neuron-derived conditioned media, either by ultracentrifugation (UC) or ultrafiltration/liquid chromatography (UF-LC). Purified EVs were quantified and characterised using transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA), and Western blotting for known exosomal markers (ALIX & CD9).

Results: Both methods produced purified EVs. NTA revealed more particles of the expected size distribution following UF-LC compared to UC. Comparative studies of morphology and marker expression will be presented.

Conclusions: We highlight the potential of UF-LC in the isolation of EVs from serum and CSF of ALS patients and confirm its utility as a potential method to advance the goal of neurochemical biomarker development in ALS.

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P45 ABERRATION OF MICRORNA EXPRESSION IN LEUKOCYTES FROM SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: microRNAs, biomarker

Background: Accumulating evidence indicates that microRNAs play an important role in the development of neurodegenerative disease. Most of the previous studies about microRNA dysregulation in Amyotrophic lateral sclerosis (ALS) have focused on the alternative expression in animal models or in limited samples from European patients. In the present study, to explore leukocytes microRNAs as a potential biomarker for diagnosing ALS, the microRNA expression profiles in Chinese ALS patients are investigated.

Method: The expression profiles of 768 human microRNAs in leukocytes obtained from 5 sporadic ALS (SALS) patients and 5 healthy controls were analyzed using microarray technology. An independent group of 42 SALS patients and 41 controls was used for validation of different microRNAs expression by real-time polymerase chain reaction assay. Area under the receiver operating characteristic curve (AUC) was used to evaluate diagnostic accuracy.

Results: Four up-expressed and seven down-expressed microRNAs were found between SALS patients and healthy controls. All the eleven microRNAs were selected for validation. Finally, 4 down-expressed microRNAs including mic-183, mic-193b, mic-451, and mic-3935 were validated in SALS. Moreover, a microRNA panel including mic-183, mic-193b, mic-451, and mic-3935 provided a high diagnostic accuracy of SALS (AUC 0.897 for the validation group).

Conclusion: This study provided the indications of abnormal microRNA expression patterns in the peripheral blood leukocytes of Chinese SALS patients. Leukocyte microRNAs provide a promising opportunity for the detection of SALS.

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P46 MIRNA EXPRESSION DIFFERENCES IN EXOSOMES FROM IDENTICAL TWINS WITH THE C9ORF72 REPEAT EXPANSION DISCORDANT FOR ALS

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Keywords: C9orf72, microRNA, exosomes

Background: Diagnosing ALS still relies heavily on clinical examination and electro-diagnostics to rule out alternative disorders with similar presentation. Several diagnostic biomarker candidates have been proposed. However, none of these have successfully translated into clinical practice. Recent research has shown that microRNAs have remarkable potential as diagnostic biomarkers. These small molecules are transcribed from non-protein coding regions of the genome. MicroRNAs are secreted by various cell types and, unlike most mRNAs, are stable in circulating body fluids due to vesicular protection in exosomes.

Objectives: In order to assess whether there are microRNAs in the cerebrospinal fluid (CSF) that are sensitive to the diagnosis of ALS, we characterized the microRNA expression profile in exosomes from CSF of identical twins with C9orf72 expansion but discordance for ALS.

Methods: CSF was obtained from the 58-year-old ALS patient with C9orf72 mutation and her unaffected identical twin. Exosomes were isolated from CSF using a commercially available kit. RNA extraction was followed by real-time PCR amplification in 384 well plates containing a total of 752 human miRNA primer sets.

Results: Eleven microRNAs (mir-16-5p, mir-99a-5p, mir-126-3p, mir-136-5p, mir-223-3p, mir-361-5p, mir-376c-3p, mir-1260a, mir-1912, mir-1972, mir-2110) were down-regulated in the CSF of the ALS patient as compared to the healthy twin. Eight microRNAs (mir-29c-3p, mir-30c-5p, mir-34b-3p, mir-93-5p, mir-194-3p, mir-219a-5p, mir-497-5p, mir-605-5p) were up-regulated in the CSF of the ALS patient as compared to the healthy twin.

Discussion and conclusions: We have identified two sets of microRNAs that were differentially expressed in the CSF of an identical twin pair with C9orf72 expansion but discordance for ALS. We found eleven microRNAs to be down-regulated and eight microRNAs to be up-regulated in the twin that has ALS as compared to the healthy twin. To our knowledge, none of the microRNAs described here have been previously identified as being altered in ALS. Our results will need to be validated in other patients with C9orf72 as well as other genetic and sporadic cases of ALS. The microRNAs identified here can be used as a panel for further validation studies.

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P47 MULTI-FREQUENCY ELECTRICAL IMPEDANCE MYOGRAPHY OF THE TONGUE: A BIOMARKER FOR BULBAR DYSFUNCTION?

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Keywords: electrical impedance myography, multi-frequency, tongue

Background: Oropharyngeal abnormalities accompany motor neuron diseases for which there are limited treatment options. Reliable tools that quantify underlying dysfunction are needed for use in therapeutic trials. Electrical impedance myography (EIM) is well-suited to provide biomarker data because the method is painless and non-invasive. Existing work indicates that EIM measurements of the tongue could provide valuable information about clinical status (1, 2). EIM is commonly performed at a single 50 kHz frequency; however, disease-associated changes suggest that multi-frequency EIM could yield further information (3, 4).

Objectives: 1) to identify at what frequencies EIM may differentiate healthy from diseased tongue muscle; and 2) to establish the reliability of tongue EIM measurements at a range of frequencies.

Methods: EIM measurements of the tongue were collected from 34 healthy individuals and eight patients, six of whom had amyotrophic or primary lateral sclerosis. EIM phase (°) was analyzed at frequencies between 50 and 500 kHz. Standard descriptive statistics, including results of a Mann-Whitney test, were determined. Reliability was assessed via intraclass correlation coefficients (ICCs).

Results: Although the between group difference was significant at each frequency assessed, the distinction between the mean phase values (+/- standard error) of healthy participants (15.69 +/- 0.22°) and patients (11.67 +/- 0.64°) was most pronounced at 100 kHz ($p = 0.0013$). The ICCs for intra- and inter-rater reliability, 0.82 and 0.75 respectively, were also superior at the 100 kHz frequency.

Discussion and conclusions: EIM may discriminate between healthy and diseased muscle at frequencies greater than 50 kHz, including at 100 kHz, where the technology is also reliable. Further work is needed to

validate these preliminary findings and to explore the role of EIM as an index of bulbar dysfunction and motor neuron disease progression.

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P48 COMPARISON OF NORMALIZATION METHODS IN THE ANALYSIS OF MAXIMUM VOLUNTARY ISOMETRIC CONTRACTION (MVIC) IN THE EMPOWER TRIAL

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Keywords: HHD, trials, outcomes

Background: Hand-Held Dynamometry (HHD) is commonly used to measure MVIC in different muscles as an outcome in ALS trials. Different analysis methods were tested to estimate the longitudinal changes in MVIC in the ALS population.

Objective: To compare different MVIC analysis methods using the EMPOWER dataset[1].

Methods: Data from the EMPOWER Phase III clinical trial were used to estimate longitudinal changes in MVIC over 12 months. MVIC data were analyzed in 3 different ways: (1) Raw MVIC: tracking percent change of MVIC overtime; (2) Normalized MVIC: as the percent predicted MVIC compared to expected normal using published normalizing algorithms by Bohannon et al.[2] for healthy volunteers; (3) Unadjusted Megascore Slope: as the sum of Z-scores for each muscle; (4) Adjusted Megascore Slope: as the sum of Z-scores for each muscle adjusted for age, gender, and weight. Assuming a 2-arm placebo-controlled trial (1-sided alpha 0.05, power 80%), we estimated the required sample size needed to detect a 20% treatment effect using MVIC, Slow Vital Capacity (SVC), and the Revised ALS Functional Rating Scale (ALSFRS-R).

Results: 942 patients enrolled in the EMPOWER Trial with longitudinal HHD, ALSFRS-R, and SVC collection over 12 months. The slope of percent change in Raw MVIC from baseline was relatively linear (Mean: -3.42, SD: 4.3). The Normalized MVIC percentages based on predicted normal values resulted in overcorrection of MVIC, for example female and older subjects had higher percent muscle strength. The age-, gender-, and weight-Adjusted Megascore was almost identical to the Unadjusted Megascore. The R^2 in regression models including age, gender, and weight were very small, ranging from 0.02 (shoulder flexion) to 0.08 (hip flexion) suggesting correction for these factors has no impact on MVIC scores. The sample size estimations for a hypothetical ALS trial were as follows: Percent change in raw MVIC (483 subjects/arm), percent predicted MVIC (>468 subjects/arm), Megascore slope (292 subjects/arm), percent predicted SVC (371 subjects/arm), and ALSFRS-R score (222 subjects/arm).

Discussion: The percent change in raw MVIC over time is linear and clinically meaningful. Using published algorithms to estimate the percent predicted MVIC for age, gender, and weight probably overcorrects and does not improve statistical power. Calculating Z-scores for ALS patients based on normal values obtained from healthy volunteers is not ideal as these two populations differ with respect to muscle strength. Based on the methods explored, ALSFRS-R remains a more sensitive outcome measure, requiring smaller samples sizes to power ALS trials compared to MVIC and SVC.

Acknowledgement: This study was funded by Biogen.

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P49 MODELING EMPOWER DATA TO IMPROVE THE EFFICIENCY OF ALS PHASE II/III CLINICAL TRIALS

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Keywords: prediction models, clinical trial design

Background: Current ALS trial designs require large sample sizes (500 subjects) in order to be powered to detect a 20% clinical effect. This is mainly due to disease heterogeneity and lack of surrogate biomarkers. We performed a series of analyses using the EMPOWER dataset[1] to explore methods to reduce the required sample size in future trials.

Objectives: (1) To determine a set of baseline variables that strongly predict disease progression; (2) to assess the impact of lead-in pre-treatment period; and (3) eligibility criteria on trial efficiency.

Methods: Data from the EMPOWER trial were used to estimate and compare the ability of multiple variables acquired over the first 3 months to predict Month-12 ALSFRS-R and Joint Rank scores. Variables with the highest predictive values were selected by Random Forest and Penalized Regression models. Predicted sample size reductions after adding the four most predictive variables were calculated using Mean Squared Error (MSE). Finally, we estimated the impact of modifying study eligibility criteria on sample size reduction assuming a hypothetical 30% treatment effect.

Results: The four most predictive baseline variables identified by Random Forest were: the slope of ALSFRS-R at baseline, Slow Vital Capacity (SVC) score, Sniff Nasal Inspiratory Pressure (SNIP) values, and Disease Duration. The four most predictive baseline variables in the Penalized Regression model were the same, except for Disease Duration, which was replaced by baseline ALSFRS-R Score. Adding these four top predictors from the Penalized Regression model results in a 14% (outcome: Month-12 ALSFRS-R) and 24% (outcome: Month-12 Joint Rank) reduction in the sample size required to detect a 30% treatment effect. There was no reduction in MSE detected, when 3-month lead-in data were incorporated, using three different models to predict ALSFRS-R or Joint Rank Score. Requiring that disease duration be $\leq$ 18 months at inclusion results in a 13% reduction in the estimated sample size, but loss of 36% of eligible subjects in EMPOWER. Eligibility criteria requiring SVC 90% results in 22% reduction of the estimated sample size, but loss of 50% of eligible subjects in EMPOWER. Finally, requiring baseline ALSFRS-R slope 0.33 points/month at inclusion results in 20% reduction in sample size, but 25% loss of eligible subjects in EMPOWER.

Discussion: Our results suggest lead-in trial periods delay treatment onset and provide negligible additional predictive value in ALS trials, and that including several parameters in the pre-specified statistical analysis models: baseline ALSFRS-R slope, ALSFRS-R score, SVC, SNIP, and disease duration results in 14-24% reduction in the required sample size. Inclusion criteria of disease duration $\leq$2 years and SVC 60% provide a good balance between reducing heterogeneity without slowing trial enrollment.

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P50 SERUM CREATININE IS A RELIABLE MARKER FOR DISEASE SEVERITY AND PROGNOSIS IN AMYOTROPHIC LATERAL SCLEROSIS: NEW EVIDENCE BASED ON INTRINSIC BRAIN ACTIVITY STUDY

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Keywords: serum creatinine, ALFF, resting-state fMRI

Background: In recent years, more and more studies have found that serum creatinine is a reliable marker to evaluate disease severity and prognosis in amyotrophic lateral sclerosis (ALS) (1). Our previous research found increased amplitude of low-frequency fluctuation (ALFF) values, which means a compensatory mechanism, in the right parahippocampal gyrus, left inferior temporal gyrus, left anterior cingulate gyrus, right superior frontal gyrus, and left middle occipital gyrus in the ALS patients using Resting-state functional magnetic resonance imaging (Rs-fMRI) (2). However, the correlation between serum creatinine and brain spontaneous compensatory activity is poorly understood.

Objective: To provide new evidence for the opinion that serum creatinine is a reliable marker for the disease severity of ALS based on correlation analysis between brain spontaneous activity and serum creatinine.

Methods: ALFF values of 5 brain regions in 22 definite ALS patients who were a part of our previous study (2) were used to perform Pearson correlation analysis with the serum creatinine. A two-tailed p-value of <0.05 was deemed significant.

Results: Compared to healthy controls, the serum creatinine level positively correlated with ALFF value in the regions of right parahippocampal gyrus (r=0.514, p=0.014), left inferior temporal gyrus (r=0.469, p=0.028) and right superior frontal gyrus (r=0.506, p=0.016).

Discussion and conclusions: The present research discussed baseline serum creatinine on a viewpoint of brain spontaneous activation. We found baseline serum creatinine levels positively correlated with ALFF value in the regions of parahippocampal gyrus, temporal and frontal gyrus, which means that baseline serum creatinine might imply the brain compensatory mechanism in early stage of disease. Above all, serum creatinine could be an important biomarker to predict disease severity and prognosis in ALS.

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P51 IMPAIRED MUSCLE UPTAKE OF CREATINE IN SPINAL AND BULBAR MUSCULAR ATROPHY

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Keywords: SBMA, serum creatinine, creatine transporter

Objective: Serum creatinine (Cr) concentrations decrease with the progression of spinal and bulbar muscular atrophy (SBMA), a hereditary disorder resulting from the degeneration of motor neurons and skeletal muscles. This study investigated the pathophysiology underlying decreased serum Cr concentrations in SBMA.

Methods: This study included subjects with SBMA (n = 52), amyotrophic lateral sclerosis (ALS) (n = 24), myopathies (n = 12), and healthy controls (n = 22). Assessments included functional parameters of motor function and muscle mass measured with dual-energy X-ray absorptiometry (DXA). We also examined the serum Cr and creatine concentrations, intramuscular creatine concentration, and skeletal muscle expression of the creatine transporter protein using immunoblotting.

Results: In subjects with SBMA, serum Cr concentrations correlated well with functional parameters such as ALSFRS-R ($r = 0.339$, $p = 0.014$) and grip power ($r = 0.546$, $p < 0.001$), and with appendicular lean soft tissue (ALST) mass in DXA ($r = 0.364$, $p = 0.008$). Both serum Cr and muscle creatine concentrations were lower in SBMA than in ALS ($p < 0.001$ and 0.049, respectively), although ALST was similar between these groups. Furthermore, muscle creatine transporter expression was decreased in SBMA compared with that in ALS, providing a molecular basis for decreased intramuscular creatine in SBMA.

Conclusion: These results suggest that myogenic defects impair muscle creatine uptake and thus contribute to motor dysfunction in SBMA. Given that creatine serves as a skeletal muscle energy source, restoring muscle creatine uptake may represent a possible therapeutic approach for SBMA.

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P51A FUNCTIONAL SCALE FOR SPINAL AND BULBAR MUSCULAR ATROPHY: CROSS-SECTIONAL AND LONGITUDINAL STUDY

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Keywords: functional rating scale, neuromuscular disease, validation

Background: Spinal and bulbar muscular atrophy (SBMA), also known as Kennedy's disease, is an adult-onset, hereditary neuromuscular disease characterized by muscle atrophy, weakness, contraction fasciculation, and bulbar involvement. There is currently no effective treatment to slow the progression of SBMA. Although clinical trials of potential therapies have been done, definite efficacy has not been clearly demonstrated in randomized controlled trials. These results appear to be partly attributable to the absence of established outcome measures that are sensitive to the disease-specific symptoms, as well as the limited number of patients, which may diminish statistical power. The progression of SBMA is slow, and existing outcome measures such as those for amyotrophic lateral sclerosis (ALS) are not sensitive enough to detect the deterioration of motor function in the patients.

Objectives: The present study aimed to develop and validate a disease-specific functional rating scale for SBMA (SBMAFRS) and to evaluate whether this scale is more advantageous than other existing outcome measures.

Methods: The SBMAFRS was designed as a quantitative outcome measure of global disability in SBMA, especially for use in clinical trials. In addition, for future use in global clinical studies, the English version of SBMAFRS was also evaluated for reliability. First, we examined the Japanese version (SBMAFRS-J) in 80 Japanese SBMA subjects to evaluate its validity and reliability. We then assessed this scale longitudinally in 41 additional SBMA subjects.

Results: The total score of the SBMAFRS-J was distributed normally without an extreme ceiling or floor effect. For SBMAFRS-J, the high intra- and inter-rater agreement was confirmed (intra-class correlation coefficients (ICCs) 0.910 and 0.797, respectively), and internal consistency was satisfactory (Cronbach's alpha 0.700-0.822). SBMAFRS-J demonstrated concurrent, convergent, and discriminant validity, except for the respiratory subscale. Longitudinally, SBMAFRS-J showed a higher sensitivity to disease progression than the existing clinical measures (Standardized Response Mean of the ALSFRS-R and the SBMAFRS-J was -0.39 and -0.48, respectively).

Next, we translated SBMAFRS-J into English to develop the English version (SBMAFRS-E) for the common use of this scale in English-speaking countries considering cultural differences by a bilingual translator. The translation

procedure followed standard methods. The SBMAFRS-E was also tested in 15 US subjects and demonstrated that the inter-rater reliability and internal consistency of SBMAFRS-E were also satisfactory (ICC of inter-rater reliability 0.837).

Discussion and conclusion: In conclusion, we developed and validated a disease-specific functional rating scale for SBMA in both Japanese and English versions, although it needs to be re-assessed in interventional studies with a larger sample size including English speaking subjects. Moreover, application of the statistical methodology such as the Rasch and item response theory should be considered for improving sensitivity of this scale.

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P52 LONGITUDINAL ASSESSMENT OF REACHABLE WORKSPACE IN ALS, USING THE KINECT SYSTEM

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Keywords: outcome measures, disease progression, clinical trial

Background: As a novel upper extremity functional outcome measure, the Kinect sensor-based reachable workspace has recently demonstrated its usefulness in ALS. Based on standardized upper limb motions, an individuals 3D reachable workspace can be reconstructed and a relative surface area (RSA) calculated. We have previously demonstrated that reachable workspace RSA, particularly the upper outer quadrant is reduced in individuals with ALS compared to normal controls, and can serve as a measure of ALS severity, correlating with the upper extremity items on the ALS Functional Rating Scale revised (ALSFRSr). Here, we present longitudinal data on reachable workspace RSA in an ALS cohort. The results suggest that Kinect-acquired RSA measures will be a reliable and sensitive outcome measure for use in ALS clinical trials.

Objectives: To assess the reliability of reachable workspace RSA in an ALS study cohort. To determine the change in RSA in an ALS cohort over 1 year, and to compare RSA to other common measures of disease progression including ALSFRSr, forced vital capacity (FVC) and manual muscle testing (MMT).

Methods: We obtained the RSA of 10 ALS subjects every 3 months for 1 year. Subjects arm movements were recorded in 4 horizontal and 4 vertical planes using a Kinect single camera system. Subjects were seated, and standardized arm movements were recorded taking 1 minute per arm. ALSFRSr, FVC and MMT were also measured.

Results: All 10 ALS subjects performed testing at each visit. Baseline RSA was 0.04- 0.80, representing severe impairment to normal function (correlating to ability to cover 4-80% of frontal reachable workspace hemisphere respectively). The upper outer quadrant RSA declined significantly over a year ($p = 0.0047$). The slope of the decline of the quadrant was 0.03848, which represented an approximate reduction of 23% in a year. Total RSA did not change significantly ($p = 0.109$) in this small series.

Discussion and conclusions: Reachable workspace decreases over time in ALS, reflecting deteriorating arm function. Using a simple, unobtrusive, sensor-based method to quantitatively measure upper extremity function in an ALS population may provide several advantages. Our method is quick and simple, reducing patient evaluation burden. Reachable workspace RSA is a continuous variable with higher granularity to detect change when compared to traditional ALS outcome measures. RSA is a highly sensitive and reliable measure. In this study, our system captured a decline of 23% in RSA within a quadrant over a year. We believe that the Kinect measured RSA holds promise to supplement or in some situations replace commonly used outcome measures due to its ease of administration and its potential for future development as a remote assessment tool within the subjects home environment.

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P53 TGF-BETA PARALLELS SMAD EXPRESSION IN ALS MUSCLE AND IS A MARKER OF DISEASE PROGRESSION

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Keywords: Smads, TGFb, muscle biomarker

Background: We recently identified Smads1,5 and 8 as muscle biomarkers in patients with ALS (1). In the ALS mouse, these markers increase over time suggesting they can track disease progression. Smads are signal transducers and become activated when members of the TGFβ superfamily engage with cell surface receptors. Once activated, the Smads translocate to the nucleus and modulate gene expression through transcriptional and miRNA pathways.

Objective: We sought to characterize potential TGFβ ligands in ALS muscle that may be linked to Smad induction and activation.

Methods: RNA sequencing data of human ALS muscle biopsy samples were reviewed for significantly increased TGFβ receptor ligands. Candidate targets were validated by qRT-PCR in a large cohort of ALS and control muscle samples and in the G93A SOD1 mouse. Protein

expression was evaluated by Western blot, ELISA and immunohistochemistry. Cultured C2C12 muscle cells were used to assess Smad activation and induction by TGFβ ligands.

Results: TGF-β1,2 and 3 mRNAs were significantly increased in ALS muscle samples compared to controls and correlated strongly with Smad1,5 and 8 expression. TGF-β1 and 3 mRNA levels correlated with MRC grade of the biopsied muscle. In skeletal muscle from the G93A mouse, TGFβ ligands were also elevated and increased with disease progression. TGF-β1 immunoreactivity was detected in mononuclear cells adjacent to muscle fibers, especially those showing atrophy. In cultured muscle cells, each TGFβ ligand was capable of activating and upregulating Smad1, 5 and 8.

Discussion and conclusions: TGF-β1, 2 and 3 are biomarkers of ALS in skeletal muscle. Their temporal expression patterns in the ALS mouse parallel those of Smad1, 5 and 8 and indicate they too are markers of disease progression. These ligands are capable of upregulating and activating Smad1, 5 and 8 in muscle cells and thus may contribute to the Smad signaling axis in ALS muscle

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Theme 4 Imaging and Electrophysiology

P54 BIOMARKERS SAVE TIME, EXPLAIN MODE OF ACTION, AND ADD SAFETY IN LONG-TERM INDIVIDUALIZED G-CSF COMPASSIONATE USE FOR ALS PATIENTS

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Keywords: biomarker, filgrastim, stemcell-therapy

Background: Granulocyte-colony-stimulating factor (G-CSF, filgrastim) is a long-term established and safe hematopoietic growth factor and may potentially compensate rapid neuronal loss in ALS by its neuroprotection, increased neurogenesis and neuronal differentiation potential. Treatment development in ALS needs validated biomarkers. Neuroinflammation is a strong contributor in ALS pathogenesis. Inflammatory cells and cytokines are known to be important co-factors in disease progression. Hematopoietic bone marrow stem cells are directly involved in G-CSF treatment, their differentiation markers may serve as additional biomarkers and also help understand G-CSF's mode of action. Measurement of pyramidal tract integrity by MRI FAI (Fractional Anisotropy) and estimation of active motor units by neurophysiology (MUNIX, eg hypothenar muscle) as downstream markers of potential therapy effects are desirable biomarkers or surrogate markers. MRI FAI studies in ALS patients have already demonstrated sensitivity to disease progression, MUNIX correlates well with disease clinical courses.

Objectives: To initiate very long-term compassionate use of G-CSF as well as longitudinal follow up of ALS patients and potential biomarkers, and present data from retrospective data analysis as a basis for further consideration.

Methods: 23 ALS patients (15 male, 8 female, mean ALS-FRS-r at start 36.75) were treated with G-CSF (150-720 MioIU/month s.c.) plus standard therapy after informed consent. Application modes were individually adapted. Monthly visits with ALSFRS-r, clinical chemistry, and bone marrow mobilization parameters were performed. Pyramidal tract integrity by MRI FAI, quantification of motor units by improved Motor Unit Number Index (i-MUNIX), and functional/differentiation markers for stem cells were obtained as biomarkers every 3 months during long-term treatment (up to 4.5 yrs).

Results: Except for mild to moderate bone pain, safety and compliance were excellent and G-CSF was well tolerated. G-CSF resulted in effective hematopoietic stem cell mobilization as determined by an increase in CD^{34+38-} stem cells. Disease progression (ALSFRS-r-decline) correlated significantly ($p<0,0001$) with i-MUNIX-decline, and with FAI-decline ($p<0,0005$). During disease progression patients with lower ALSFRS-r mobilized fewer monocytes ($p=0,048$) and CD^{34+38-} stem cells ($p=0,037$), but more eosinophils ($p=0,002$). We found colony forming capacity for bone marrow differentiation under G-CSF treatment to be non-significantly associated with survival. In retrospective analysis we found a significantly lower ALS progression rate ($p<0,0001$) and a clinically relevant prolongation of overall survival ($p<0,0001$) in long-term G-CSF treated ALS patients compared to the current PRO-ACT database.

Discussion and conclusions: Long-term administration of G-CSF in ALS patients is safe and feasible. Quantitative surrogate markers for upper and lower motor neuron integrity (DTI FAI and i-MUNIX) as well as cellular markers, namely bone marrow differentiation, monocytes and eosinophils, are promising biomarkers for ALS treatment development. As data are robust, a prospective clinical trial is urgently needed.

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P55 CAN A MRI-BIOMARKER PROVIDE STABILIZATION IN G-CSF-TREATED ALS PATIENTS?

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Keywords: DTI, FAI, biomarker

Background: G-CSF (Granulocyte Colony Stimulating Factor, filgrastim) may modulate clinical progression of patients with motor neuron disease. To elucidate potential mechanisms of action of such modulation longterm individual follow-up DTI (diffusion tensor imaging) was applied in ALS patients treated with G-CSF in an open label compassionate use program.

Methods: 23 patients (15 male, 8 female, mean age 51,4) received individual G-CSF long-term continuous treatment up to 48 months in an outpatient setting after informed consent. For safety and feasibility, cranial MRI with calculation of FA (fractional anisotropy) for upper motor neuron integrity and other clinical biomarkers were obtained every 3 months. Between 2 and 16 image-datasets per patient were obtained and retrospectively analyzed, two consecutive 1.5 Tesla Siemens scanners were employed. Results were referenced to neurophysiological MUNIX (Motor Unit Number Index) as indicators for lower motor neuron function and clinical (ALSFRS-r) values.

Results: The baseline and follow up values for the selected biomarkers can be visualised along with their change over time. We could trace the individual path of FA decline for each patient. This decline per year correlated significantly not only in group comparison, but also on individual levels with the clinical disease progression (ALS-FRS-r). We also detected a significant correlation between FA decline per year and MUNIX loss per year (p<0,001).

Conclusions: FA is a very promising and precise MRI-biomarker in ALS. It is helpful in staging, evaluation of clinical progression, treatment efficacy, safety, as well as in understanding mode of action in restorative treatment approaches. FA - unaffected by individual genetic pathophysiology - needs to be validated as a robust, independent biomarker to help accelerate clinical trial results in ALS patients.

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P56 QUALITY CONTROL OF MULTICENTER, HIGH RESOLUTION T1 MRI AS A BRAIN BIOMARKER IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: MRI, multicenter trial, quality control

Introduction: In ALS, pathology spreads within the brain along tracts which are connected to the primary motor cortex, eventually causing atrophy in several cortical areas. Grey and white matter signals in high resolution T1 MRI can be analyzed using cortical thickness analyses, voxel-based morphometry (VBM), voxel-based intensitometry (VBI), deformation based mapping and other T1 tools. These tools are capable of detecting ALS related pathology in groups of patients. In order to utilize these capabilities as a monitoring and exploration tool in large multicenter cohorts, the quality of T1 datasets must be comparable between different scanners and centers. Causes of low quality scans must be identified and corrected for by changing acquisition, by using software correction, or both.

Objectives: (i) to quantify the systematic differences in high resolution T1 scans in a large cross sectional data set uploaded to the central MRI repository of the Neuroimaging Society in ALS (NiSALS); (ii) to identify the major components contributing to the quality of the scans; (iii) to correct for scanner specific systematic distortions and (iv) define a set of quality markers to allow pooling of data for large multicenter trials.

Methods: 500 T1 data sets were uploaded from 20 centers in Europe, the USA and Canada. Data sets were analyzed using preprocessing from the VBM12 package within SPM on the Matlab platform. A custom developed set of quality markers (Dahnke and Gaser) was used to describe geometrical and noise distortions in a quantitative manner and correct for these distortions on a per scanner basis. Mahalanovis distance analyses were used to describe the relative quality of the scans within the NiSALS cohort, and in comparison to other freely available cohorts.

Results: Data from 15 centers passed the minimum requirements for pooling using stringent criteria, data from three more centers were allowed to enter when correction measures were finely adjusted. The quality of the NiSALS data set was comparable to the ADNI data set which imposed strict measures during data acquisition.

Conclusion: Quality control of T1 data sets in a centralized repository allows the pooling of large MRI data sets from many different centers, the quantification of scanner specific distortions, the software correction of some of these distortions, and the monitoring of the quality of T1 data as it would be used during a clinical trial.

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P57 MRI CORRELATES OF (11C) PBR28 PET AS A BIOMARKER FOR ALS

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Keywords: MRI, PET, Inflammation

Objectives: To characterize changes in gray (GM) and white matter (WM) microstructures in relation to (^{11}C)-PBR28 binding in people with Amyotrophic Lateral Sclerosis (ALS).

Background: Diffusion tensor imaging (DTI), voxel based morphometry (VBM) and cortical thickness analysis (CTA) are brain imaging techniques that can be used to quantify the micro- and macro-structural integrity of the brain white and gray matter *in vivo*. DTI Studies in patients with ALS consistently reported decreased fractional anisotropy (FA) and increased diffusivity values along the corticospinal tracts (CST), consistent with reduced integrity of these pathways. In addition VBM and CTA studies have revealed motor cortex thinning in the same patient population. We have previously provided *in vivo* evidence for glial activation in the motor cortices and subcortical white matter using (^{11}C) PBR28 Positron emission tomography (PET) (1).

Here, we hypothesized that neuroinflammation measured by (^{11}C) PBR28-PET in the motor cortices and the CST is correlated with GM and WM changes measured by DTI and cortical thickness.

Methods: Ten ALS patients (53.2y 10.75) and 6 controls (47.3y 12.25) underwent simultaneous PET and MRI imaging using Siemens 3T MR-PET scanner. Diagnosis history and clinical assessments including upper motor neuron burden scale (UMNB), and amyotrophic lateral sclerosis functional rating scale revised (ALSFRS-R) were obtained from ALS patients. We applied voxel-based DTI and VBM by Freesurfer (5.3.0). Tract-based spatial statistics (TBSS) was employed by FSL (FMRIB Software Library V5.0.7) to investigate the WM microstructural integrity in ALS patients. Spearman correlation coefficient was carried out to study the relationship between PBR28 binding and FA in the motor cortices, and to evaluate the relationship between cortical thickness, age and the clinical scales.

Results: Our results revealed significantly decreased FA in the right and left precentral gyrus WM (right P=1.24 X 10^{-4}; Left P=6.19X10^{-5}) and in the left precentral gyrus GM (P=0.019) in the ALS group as compared to controls. This matches anatomically the areas of increased PBR28 binding in the left precentral gyrus WM (P=0.0098). Cortical thickness in the ALS group was reduced in the left precentral gyrus (P=0.0092). Reduced cortical thickness correlated with lower ALSFRS_R (Left r = 0.84; P=0.0002 right r = 0.89; P=0.0003), and longer disease duration (Left r = -0.65; P=0.039 Right r = -0.83; P=0.0031). Higher PBR28 binding correlated with lower FA in the left precentral gyrus white matter(r = −0.69; P = 0.028) and with cortical thinning in the left precentral gyrus(r = −0.75; P=0.01).

Conclusion: Our findings suggest that increased neuroinflammation co-localize with cortical thinning in the motor cortex and white matter changes in the CST in the same patient population. These findings demonstrate the power of advanced neuroimaging in demonstrating the link between a disease mechanism (inflammation) and anatomical changes (cortical atrophy and white matter changes in the CST).

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P58 INCREASED FUNCTIONAL CONNECTIVITY WITHIN THE SENSORIMOTOR RESTING-STATE NETWORK IN ALS: A CANDIDATE MEG-BASED BIOMARKER

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Keywords: MEG, biomarker, neurophysiology

Background: Alteration in cortical functional connectivity (FC) is a candidate for a sensitive marker of neurodegeneration in ALS. Resting-state functional MRI (fMRI) studies have described bi-directional FC alterations in ALS within motor-related networks. Resting-state networks (RSNs) may be characterised through power fluctuations of oscillatory neuronal signals measured using magnetoencephalography (MEG), bypassing potential confounds of the fMRI hemodynamic response.

Objectives: To characterise the impact of ALS on MEG generated RSNs.

Methods: MEG was recorded in the resting-state in 10 ALS patients and 10 age-matched controls. Structural MRIs were acquired for co-registration of MEG data to individual anatomy. RSNs were extracted within a frequency envelope of 4-30 Hz by independent component analysis from beamformed data, and groups were contrasted using a dual-regression approach, statistically appraised by cluster permutation testing.

Results: ALS patients demonstrated *increased* FC within the sensorimotor network, significant within a cluster incorporating the right precentral gyrus (p = 0.046).

Discussion and conclusions: Evidence that ALS pathophysiology is characterised by increased FC is reproducible using MEG. This further highlights FC as a sensitive biomarker with potential mechanistic relevance, either through excessive excitatory neurotransmission, or through a deficit in cortical inhibition. Normalisation of FC in future therapeutic trials is a candidate outcome measure.

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P59 THE CANADIAN ALS NEUROIMAGING CONSORTIUM (CALSNIC)

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Keywords: Biomarker, MRI

Background: Prior studies support the potential of several advanced MR modalities as biomarkers of disease progression, survival, and therapeutic effect. However, these studies have had limited impact due to several factors including variably small and heterogeneous samples and variable methodologies. Furthermore, studies have been reported from single centres, resulting in biased cohorts with reduced generalizability of the findings to the broader population. In response to this, the Canadian ALS Neuroimaging Consortium (CALSNIC) was established to conduct a prospective multicenter biomarker validation study. Key objectives are to identify the relationship of MR indices with disease progression and clinical subtypes.

Methods: A multimodal MR protocol consisting of 3DT1, diffusion tensor imaging, magnetic resonance spectroscopy, and resting state functional MRI was standardized across 5 sites (University of Alberta, University of Calgary, Western University, University of Toronto, McGill University) on scanners operating at 3 Tesla. These imaging sequences were chosen with accompanying image processing pipelines that permit *in vivo* interrogation of pathological elements relevant to ALS, including neuronal and white matter integrity, gliosis, and structural and functional connectivity. Physical exam, cognitive, and post-mortem protocols were also standardized across centres to permit clinical and pathological correlations with MR metrics.

Results: Harmonization of MRI, clinical, and post-mortem protocols was completed across sites in 2014. Travelling head data was acquired to assess inter- and intra-site variability. At the time of writing four sites were operational with 10 patients and 7 healthy controls enrolled.

Discussion: CALSNIC is a multidisciplinary research platform evaluating potential MRI biomarkers of neurodegeneration. It has laid the foundation for collaborative clinical and translational research on a national and multinational level.

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P60 A CONNECTIVITY-BASED ANALYSIS OF FRONTOSTRIATAL AND CORTICO-BASAL NETWORKS IN ALS

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Background: Basal ganglia pathology is increasingly recognised in ALS. The heterogeneous clinical symptoms of ALS are manifestations of complex network dysfunction as opposed to isolated grey or white matter pathology. Dysfunction of frontostriatal, nigrostriatal, and corticobasal networks contribute to the unique cognitive, behavioural, pyramidal and extrapyramidal deficits observed in ALS. Recent neuroimaging studies have demonstrated significant subcortical grey matter involvement in ALS, but relatively little is known of the selective vulnerability of striatal nuclei.

Objectives: The aim of this study is to comprehensively characterise striatal and sub-thalamic networks in ALS based on probabilistic cortical-striatal connectivity profiles.

Methods: Eighty *C9orf72* negative ALS patients, twelve patients carrying the *C9orf72* hexanucleotide repeat and forty age-matched healthy controls were included in a multi-parametric neuroimaging imaging study. Subcortical structures were delineated based on high resolution 3D structural data sets, using intensity gradients and automated boundary corrections. Pathology within the structures was evaluated using connectivity-based segmentation. Seven sub-regions were evaluated according to cortical-striatal anatomical connections. The cortical targets included: limbic; executive; rostral-motor; caudal-motor; parietal; occipital; and temporal cortical zones. Both diffusivity and density analyses were carried in the identified sub-nuclear regions. Additional seed-based rfMRI analyses were carried out using the above segmentation.

Results: The most significant diffusivity and density alterations were identified in thalamic and caudate foci which connect to caudal motor regions. Pathological changes were also mapped to striatal nuclei connecting to rostral motor areas. Hexanucleotide repeat carriers showed considerable pathology in limbic and executive projections. The connectivity-based pathology profile of hexanucleotide repeat carriers is distinctly different from *C9orf72* negative patients.

Discussion and conclusions: Striatal pathology in ALS exhibits network-wise vulnerability mirroring cortical atrophy patterns. Comprehensive basal ganglia analyses demonstrate connectivity based susceptibility patterns. Our findings support the notion that interconnected brain regions show concomitant neurodegeneration and supports pathophysiological observations of connectivity-based disease spread.

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P61 WHOLE-BRAIN FUNCTIONAL CONNECTIVITY CHANGES IN CLASSICAL ALS ARE RELATED TO PHYSICAL DISABILITY

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Keywords: MRI, functional connectivity, ALSFRS-R

Background: Previous studies on disease related connectivity changes in ALS show a relationship between structural integrity and disease progression (1) and pTDP-43 pathology (2). However, studies investigating the relationship between functional connectivity changes and the progression of the disease revealed rather inconclusive results.

Objectives: To map whole-brain functional connectivity changes associated with patients' physical disability using voxel-level graphs.

Methods: 64 patients with ALS and 38 healthy controls underwent resting-state fMRI at 3T (TR = 2200ms, isotropic voxel size = 3.5mm3). Subject-specific connectivity graphs were constructed by defining gray matter voxels as nodes, and establishing weighted edges by estimating internodal functional connectivity between the nodes' associated time-series. In order to assess between-group differences, edge-level t-statistics were computed across groups (FDR < 0.05). Edge-level correlations with the patients' ALSFRS-R score were computed across graphs to assess connectivity changes associated with physical severity.

Results: ALS-related decreases in connectivity were found within the sensori-motor system and the temporo-occipital lobe. Reduced connectivity within the bilateral sensorimotor cortices correlated with higher physical disability, whereas the relationship between connectivity reductions in the temporo-occipital changes and higher physical disability was limited to the bilateral hippocampi. Increased connectivity related with ALS was found between fronto-parietal and parietal-temporal regions. Connectivity increases associated with increasing disability were observed between the frontal cortices and bilateral parietal cortex, the basal ganglia, and the inferior temporal cortex. The parietal cortices also exhibited increased connectivity correlated with disability with the occipito-temporal cortex, basal ganglia and amygdala.

Discussion: ALS progression is accompanied by concurrent reductions in motor-related connectivity. Changes in hippocampal connectivity were also correlated with disability, suggesting a key functional role of the hippocampus in disease progression (3). With higher disability, patients exhibited increased connectivity across the frontal and parietal cortices, possibly reflecting a loss of cortical inhibition. Strinkingly, large patterns of ALS-related connectivity decreases in the temporo-occipital were not modulated by the patients' physical disability, indicating that changes occur really early during disease pathology.

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P62 NEURITE ORIENTATION DISPERSION AND DENSITY IMAGING (NODDI) DEMONSTRATES MICROSTRUCTURAL CHANGES ASSOCIATED WITH AMYOTROPHIC LATERAL SCLEROSIS

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Background: There is limited insight into the alterations within complex structures such as gray matter neuropil that accompany amyotrophic lateral sclerosis (ALS). Imaging studies focusing on the microstructural alterations underlying ALS are lacking.

The organisation and density of neurites can be assessed using NODDI (Neurite Orientation Dispersion and Density Imaging), and altered architecture quantified. (1) NODDI has demonstrated specificity at localising abnormalities in various disease states, (2) as well as gray matter (GM) alterations associated with aging (3).

We used NODDI to investigate the microstructural abnormalities accompanying ALS and assess the contribution of underlying mechanisms, such as loss of myelinated axons versus altered dendritic complexity.

Objectives: Apply NODDI to ALS in order to: evaluate how the neurodegenerative process affects the neuronal density in GM and the white matter (WM); visualise themicrostructural architectural changes *in vivo* to ascertain how damage to the neuropil region relates to axonal degeneration.

Methods: Diffusion-weighted and resting state fMRI scans were obtained on 12 ALS participants and 14 healthy controls of similar age range.

Quantitative measures of neurite orientation dispersion index (ODI), neurite density index (NDI), and Isotropic compartment (ISO) were related to Fractional Anisotropy (FA).

Results: FA was lower in the ALS group compared to controls within the cortico-spinal tract and primary motor cortex, predominantly on the right side (p= 0.116). NDI was reduced in the ALS group throughout the cortico-spinal tracts and primary motor cortices bilaterally (p=0.086). There were cortical GM regions as well as subtle areas in the cortico-spinal tracts where ODI was reduced in the ALS group (p=0.009) ISO was increased in the ALS group compared with controls within the ventricles and sulci (p=0.045).

Discussion: NODDI is an established MRI technique for estimating the microstructural complexity of dendrites and axons (1-3). ALS is associated with loss of neurite density in both the highly myelinated structures of the cortico-spinal tracts and the motor cortices. NDI was more sensitive than FA at detecting group differences. Reduced neurite orientation dispersion within cortical GM regions may represent dendritic pruning. Increased isotropic component within ventricles and sulci is likely to reflect ALS related atrophy. NODDI demonstrated higher sensitivity and specificity to DTI at characterising the neurodegenerative process associated with ALS.

Conclusion: NODDI provides an insight into the neurodegenerative process occurring in ALS *in vivo* and demonstrates the potential to fulfil a major role as an imaging biomarker for ALS.

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P63 THE SELECTIVE ANATOMICAL VULNERABILITY OF ALS - DISEASE-DEFINING AND DISEASE DEFYING BRAIN REGIONS

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Keywords: MRI, biomarker, pathology

Background: Neuroimaging in ALS has gained significant momentum in recent years highlighting ALS-specific pathology in early stage and presymptomatic disease. While imaging studies invariably highlight motor cortex, corpus callosum and corticospinal pathology, recent studies have demonstrated extensive frontotemporal changes, and cerebellar and basal ganglia pathology. With the emergence of automated classification methods, the identification of unaffected brain regions has become equally important. The quantitative evaluation of these anatomical locations enables the discrimination of ALS data sets from disease controls.

Objectives: The aim of this study is to highlight key basal ganglia nuclei, cortical grey matter regions and white matter tracts which are not affected by ALS, even in advanced disease.

Methods: Hundred and twenty ALS patients and eighty age-matched healthy controls participated in a multi-parametric neuroimaging imaging study. Subcortical structures were evaluated based on high resolution 3D structural data sets, using intensity gradients and automated boundary corrections. Grey matter analyses were carried out using both cortical thickness analyses and voxel-based morphometry. Alterations in white matter integrity were assessed based on diffusion tensor imaging data using several diffusivity parameters. Network integrity was evaluated by fMRI data acquired in the resting state.

Results: While the cortical signature of ALS includes considerable precentral gyrus, orbitofrontal, and anterior cingulate pathology, the posterior cingulate, postcentral gyrus, occipital and parietal lobes remain relatively spared. The commissural white matter tracts of the forceps major in the splenium of the corpus callosum also remain relatively intact in sharp contrast to the striking degeneration of the genu and mid-body of the corpus callosum. The fornix and posterior limb of the internal capsules show disease-specific degeneration. Basal ganglia analyses also confirmed striking selective vulnerability, with the relative sparing of thalamic sensory nuclei, amygdala, putamen, pallidum, and the anterior portions of the hippocampi.

Discussion and conclusions: The systematic characterisation of unaffected brain regions in ALS have important pragmatic implications for the development of classifying analyses as these brain regions distinguish ALS from mimic disorders and disease controls. Sparing of distinct, but contiguous regions raise important pathophysiological, phylogenetic and ontogenetic questions regarding ALS pathogenesis and disease spread.

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P64 GRAY AND WHITE MATTER ALTERATION IN ALS PATIENTS WITH OR WITHOUT COGNITIVE IMPAIRMENT: A COMBINED TRACT BASED SPATIAL STATISTICS AND VOXEL BASED MORPHOMETRY STUDY

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Keywords: TBSS, VBM, cognitive impairment

Objectives: The aim of the study is to evaluate the involvement of cortical and subcortical gray and white matter structures in ALS patients, with and without cognitive impairment, compared to healthy controls.

Methods: A prospective gray and white matter MRI study was undertaken with 30 ALS patients: 17 with pure ALS (pALS; male=11, female=6; median disease duration 25.99 months); 13 with ALS with cognitive involvement (ALSci; male=8, female=5; median disease duration 16.82 months); and 19 healthy controls. We acquired structural and diffusion MRI, using both voxel-based morphometry (VBM) and tract-based spatial statistics (TBSS). All patients were screened for cognitive function and behavioral profiling.

Results: Cortical and subcortical changes in ALSci patients were identified in thalamic, orbitofrontal regions and association tracts, as seen by reduced functional anisotropy (FA), increased mean diffusivity (MD) and increased Axial diffusivity (D_{axial}). In pALS, abnormalities were confined to the cortico spinal tracts and corpus callosum with limited involvement of extramotor structures. VBM analysis identified a reduction in caudate nucleus gray matter volume in ALSci compared to pALS patients.

Conclusion: Neuronal tissue damage in ALSci patients is distinct from that of pALS patients. ALSci patients are distinguished by DTI parameters highlighting degeneration in the cortical and subcortical frontotemporal cortex, in addition to the motor associated damage seen in pALS patients. The differences in the caudate nucleus between these groups can be explained by the role of caudate nucleus in cognition. This suggests that previously described extra-motor white matter abnormalities cohorts of ALS patients could be due to inclusion of ALS subjects with cognitive impairment

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P65 NEURONAL ACTIVATION OF BEHAVIORALLY IMPAIRED PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS IN TASKS OF EXECUTIVE FUNCTIONING: A FUNCTIONAL MAGNETIC RESONANCE IMAGING STUDY

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Keywords: functional magnetic resonance imaging, theory of mind, cognition

Background: A number of patients with amyotrophic lateral sclerosis (ALS) are known to exhibit behavioral impairments and/or deficits in various domains of cognitive functioning during the course of the disease. Deficits in executive functions are especially prominent. Yet, the neurophysiological substrates underlying these changes are not well understood.

Objective: To investigate neural correlates of cognitive and behavioral impairment in ALS patients using functional magnetic resonance imaging (fMRI).

Methods: We developed an fMRI paradigm consisting of two executive function tasks from the Edinburgh Cognitive and Behavioural ALS Screen (ECAS): the theory of mind task, where emotions of others have to be correctly attributed, and the alternation task, requiring subjects to alternate between numbers and letters in ascending order. 73 ALS patients and 43 age-, gender-, and education-matched healthy controls completed the ECAS first and, afterwards, the fMRI-adapted tasks in a 3T scanner. Additionally, the ALS Functional Rating Scale-Revised was administered to get a measure of patient's physical impairments.

Results: Healthy controls performed better than ALS patients on the ECAS overall ($p<0.001$) and in the alternation ($p<0.001$) but not in the theory of mind task ($p=0.297$). In the fMRI tasks, the patient cohort showed significantly more activation in the theory of mind task than healthy controls in the bilateral thalamus, left posterior cingulate cortex and left pallidum. In the alternation task more activation was found in the right superior parietal lobule as well as the right superior frontal gyrus in the patient cohort. ALS patients with behavioral abnormalities, as assessed by the carer interview of the ECAS, did not perform worse in the overall ECAS or any of its tasks of executive functioning, but showed less activation in the right superior and medial gyrus in the theory of mind task than those without such impairments. In the alternation task, more activation in the left orbital gyrus was found in ALS patients without behavioral impairments.

Discussion and conclusions: These findings suggest a compensatory process in subcortical and cortical areas for ALS patients when performing tasks of executive functioning such as theory of mind or alternation. Furthermore, patients who exhibit signs of behavioral impairment show reduced neuronal activation in executive tasks, indicating a close relationship between the domains of executive dysfunction and behavior, which can be analyzed using functional imaging techniques. This approach is thus very promising to further investigate further the non-motor impairments of ALS patients.

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P66 POST MORTEM BRAIN IMAGING TO INTERPRET THE *IN VIVO* MRI SIGNATURE OF MND

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Keywords: MRI, histopathology, validation

Background: *In vivo* magnetic resonance diffusion tensor imaging studies have demonstrated reduced fractional anisotropy and increased radial diffusivity, particularly in the CSTs and the body of the CC in ALS patients (1), and have recently been suggested to enable non-invasive disease staging related to the pattern of TDP-43 pathology in ALS (2). However, we currently have a poor understanding of the relationship between emerging *in vivo* MRI-based biomarkers and *post mortem* histopathology in MND.

Objectives: The aim of this study is the development of post mortem tractography approaches for the delineation of white matter tracts and other anatomical landmarks known to be affected in MND, in order to enable the investigation of the tissue histological correlates of MRI changes in MND.

Methods: Ultra-high-resolution structural and diffusion-weighted steady-state free precession (DW-SSFP) magnetic resonance images of *post mortem* control and MND whole brains were acquired at 7 Tesla. Tracts-of-interest for subsequent histopathology/imaging comparisons, including the corticospinal tracts (CSTs), the corpus callosum (CC), the corticopontine tract, the corticorubral tract, the corticostriatal pathway, and the proximal portion of the perforant path, were identified using probabilistic tractography as implemented in FMRIBs Software Library. Furthermore, non-diffusion MRI modalities such as quantitative T1, T2 and susceptibility maps were derived from the same tissue.

Results: Probabilistic tractography in our high-resolution post mortem diffusion data allowed the semi-automated delineation of the CSTs, the CC, the corticopontine tract, the corticorubral tract, the corticostriatal pathway, the proximal portion of the perforant path, as well as the subdivision of major white matter tracts according to their exact cortical origin (eg, primary motor hand;foot;face area representations in the CSTs and the CC), which is of highest relevance for clinical symptom correlations.

Discussion and conclusions: This work will complement on-going acquisition of histopathological markers derived from the same tissue, enabling us to explore recent concepts of staging in conjunction with clinical phenotype. While no single MRI measure will reflect (pathology in an isolated cellular compartment, we hypothesize that it will be possible to define the relative contribution (of pathological modalities to specific MR signals, thus providing a vital and hitherto missing link to the *in vivo* disease.

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P67 CORRELATION BETWEEN MRI MEASURES OF GREY AND WHITE MATTER AND CLINICAL MEASURES IN C9ORF72 SYMPTOMATIC AND ASYMPTOMATIC CARRIERS

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Background: Persons with a repeat expansion in the C9ORF72 gene have varied degrees of motor and cognitive dysfunction with characteristics of ALS and FTD. Alterations in structural brain MRI scans have previously been reported for sporadic ALS, with different findings from those reported for various forms of FTD.

Objective: The long-term goal is to determine whether imaging measures coincide, precede, or lag the onset of clinical disease. In this interim analysis we examine the relationship between MRI and clinical measures in C9ORF72 patients.

Methods: This is an ongoing natural history study of patients and asymptomatic carriers with a confirmed mutation with clinical and brain MRI measures at baseline and follow-up visits at 6- and 18-months. For this initial analysis, persons were classified as having motor impairment if meeting El Escorial criteria for possible, probable or definite ALS. Persons were classified as having cognitive impairment who had a raw score <133 on the Mattis Dementia Rating Scale (DRS-2). A GE 3T scanner was used to obtain T1-weighted images and diffusion tensor imaging (DTI), as well as T2, FLAIR, and resting state fMRI sequences. The same sequences were obtained on 30 age-matched controls. Cortical thickness and subcortical volumes were measured using the FreeSurfer software program (surfer.nmr.mgh.harvard.edu); DTI was processed using TORTOISE (https://science.nichd.nih.gov/./TORTOISE) and MRI Studio (http://cmrm.med.jhmi.edu/) software. The correlations between MRI measures and the ALSFRS-R score and Frontobehavioral Inventory (FBI) score were examined.

Results: Nineteen C9ORF72 carriers (3 pre-symptomatic) from different pedigrees completed baseline scanning; to date 14 completed 6-month and 2 completed 18-month follow-up scans. Preliminary analysis shows a significantly greater ($p<0.01$) ratio of ventricular to intracranial volume in patients at baseline when compared to healthy controls. There was a trend towards a correlation between ventricular volume and FBI scores in C9ORF72 carriers. Cortical thickness was diffusely decreased across all lobes (frontal, temporal, parietal, occipital, cingulate, and insular) bilaterally. The ALSFRS-R score correlated with pre-central cortex thickness in patients with cognitive impairment ($p<0.001$) but not in patients classified with only motor dysfunction. DTI fiber tracking showed a trend toward lower FA in the uncinate fasciculus and genu of the corpus callosum in patients

with cognitive impairment, and reduced FA of the corticospinal tract in patients with motor impairment.

Conclusion: Measures of global atrophy dominate the imaging in C9ORF72 carriers at baseline, and are mostly correlated with cognitive dysfunction. Follow up studies are being performed to see if these and other measures predict disease progression.

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P68 BASAL GANGLIA PATHOLOGY IS ASSOCIATED WITH NEUROPSYCHOLOGICAL DEFICITS IN C9ORF72-NEGATIVE ALS

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Keywords: basal ganglia, cognition, ALS-FTD

Background: The most frequently reported neuropsychological deficits in ALS include apathy, executive dysfunction, impairment in social cognition. Language and memory deficits are also increasingly recognized. The distinct neuropsychological profile of ALS points beyond isolated gray and white matter pathology and suggests complex frontostriatal circuit dysfunction, but the contribution of subcortical structures to the patients' cognitive profile has not yet been investigated.

Objectives: (1) To invesigate the contribution of basal ganglia pathology to the cognitive phenotype of ALS and evaluate the predictive value of basal ganglia measures for phenotypic classification. (2) To explore the relationship between structure-specific neuropsychological performance and basal ganglia atrophy. (3) To characterize patterns of intranuclear pathology within the basal ganglia.

Methods: 67 *C9orf72*-negative ALS patients and 39 healthy controls were included in a cross-sectional quantitative MRI study. Seven ALS patients met criteria for comorbid bvFTD (ALS-FTD); 18 patients met the Strong criteria for cognitive or behavioral impairment (ALS-Plus); 42 patients had no cognitive deficits (ALS-Nci). Volumetric, shape, and density analyses were performed for the thalamus, amygdala, nucleus accumbens, hippocampus, caudate nucleus, pallidum, and putamen. A linear discriminant analysis was performed to evaluate the predictive value of subcortical volumes for phenotypic classification. Structure-specific correlations were computed between memory scores and hippocampal volumes, and apathy scores and nucleus accumbens volumes.

Results: A preferential vulnerability has been identified within basal ganglia sub-regions, which connect directly to key cortical sites of ALS pathology. While the anatomical patterns were analogous, the degree of volumetric, shape, and density changes confirmed incremental pathology through the spectrum of ALS-Nci, ALS-Plus to ALS-FTD. Hippocampal and thalamic volume reductions were the most sensitive predictors of phenotypic classification. Performance on verbal memory tests correlated with hippocampal volumes and accumbens nuclei volumes showed a negative correlation with apathy scores.

Discussion and conclusions: We demonstrate correlations between basal ganglia measures and structure-specific neuropsychological performance. Our results indicate incremental basal ganglia pathology across the ALS – ALS-FTD spectrum, suggesting that the degree of subcortical gray matter pathology in *C9orf72*-negative ALS is closely associated with neuropsychological changes. Our findings also provide evidence of a network-wise vulnerability of interconnected cortical and subcortical gray matter regions.

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P69 SEQUENTIAL PET AND MRI SCANS IN A PATIENT WITH ALS-FTD REVEAL WORSENING BRAIN METABOLISM AND CORTICAL THINNING WITH DISEASE PROGRESSION

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Keywords: cortical thickness, hypometabolism, primary progressive aphasia

Background: Single time-point 18-fluorine-2-fluoro-2-deoxy-D-glucose (^{18}F-FDG) PET studies of patients with ALS, with or without frontotemporal dementia (FTD), have demonstrated brain regions of hypometabolism or hypermetabolism (1, 2). We recently reported that regions of cortical hypometabolism overlap structural abnormalities in brains of ALS-FTD patients and may precede them in some regions (3).

Objectives: To determine whether longitudinal PET and MRI measures in a patient with non-familial bulbar-onset ALS-FTD reveal evolving imaging pathology that corresponds to clinical progression.

Methods: High-resolution T1-weighted 1.5T MRI and ^{18}F-FDG PET images were acquired during routine clinical evaluation as previously described (3) in a 69 year old right-handed female, with bulbar-onset upper motor neuron (UMN) predominant ALS-FTD. Readings were taken on the same day at baseline (15 months after symptom onset) and 22.3 months later. Detailed

neurologic and neuropsychometric evaluations were performed within one month of imaging. At each time point, measures of cortical thickness (Freesurfer) and cerebral glucose metabolic rate (CGMr, NEUROSTAT) were obtained (3) and percent change for each, calculated.

Results: At baseline scanning, neurological deficits included lack of insight, pseudobulbar affect, severe dysphasia, dysarthria with right body predominant UMN signs and ALSFRS-R=42 (6/12/12/12); psychometric testing revealed significant expressive language problems, with mild complex attention and executive function difficulties. At follow-up scanning, neurological decline was primarily bulbar (mixed UMN and LMN signs), with preserved limb and respiratory function, although worsened ALSFRS-R=20 (3/8/4/5) with DFS=−0.99; marked decline occurred in executive functions, language (both expressive and receptive), causing aphasia and dysgraphia, all consistent with primary progressive aphasia (PPA) form/behavioral variant (bv) ALS-FTD. Worsening hypometabolism was most obvious in averaged cerebral cortex (−28%), with regional decline in left>right hemispheres: frontal pole (−9%); anterior cingulate (−9.5%); temporal (−12%); and parietal (−10%) cortices; subcortical regions showing progressively decreased CGMr included: caudate (−11%); putamen (−16%); and thalamus (−44%). Cortical thinning worsened in bilateral prefrontal lobe regions, including Broca's area (−7%), and in left>right temporal pole (−12%), superior temporal (−10%), entorhinal (−11%) and parahippocampal (−10%) cortices.

Discussion and conclusion: Clinical decline over 22.3 months in this 69/71 year old patient was accompanied by worsening cerebral hypometabolism (including in subcortical nuclei) and cortical thinning in regions known to degenerate in PPA form and bvALS-FTD. Determining relative proportions of metabolic vs. structural changes in each affected brain region will reveal their sequential progression. Longitudinal PET and MRI studies of more patients with ALS-FTD could provide insight into disease evolution and pathogenic mechanisms.

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P70 *IN VIVO* DTI SHOWS PATHOLOGY SPREADING IN ALS: STAGING ANALYSIS IN MORE THAN 300 DATA SETS

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Keywords: diffusion tensor imaging, pathology spreading, fiber tracking

Background: Neuropathological studies in ALS have shown that ALS may disseminate in a regional sequence in four disease-related patterns (1). The application of diffusion tensor imaging (DTI) to *in vivo* analysis of staging was performed by use of fiber structures that are prone to be involved at each neuropathological pattern of ALS (2) in a large scale study sample including follow-ups.

Methods*:* 414 DTI data acquired at 1.5T and at 3.0T from ALS-patients (N=289) and from controls (N=125) were analyzed by a tract of interest (TOI)-based fiber tracking approach. Tracts were analyzed that become involved during the course of ALS, ie, the corticospinal tract (stage 1); the corticorubral and the corticopontine tracts (stage 2); the corticostriatal pathway (stage 3); the proximal portion of the perforant path (stage 4), together with reference pathways. In addition, forty ALS-patients obtained a follow-up scan with a time-interval of 6 months in average.

Results: The statistical TOI-based analyses by tractwise fractional anisotropy statistics (3) showed differences between ALS-patients and controls for all investigated tracts. Data analysis at the individual level allowed for a categorization into ALS patterns, in plausible agreement with *post mortem* neuroanatomical studies; 34 patients were categorized as stage 1; 27 as stage 2; 66 as stage 3; and 87 as stage 4. Of the 40 longitudinal data sets, 4 ALS patients showed an increase in ALS stage and 31 ALS patients remained stable, while 5 patients could not be analyzed owing to artefacts caused by intolerance to the scanning situation.

Discussion: In this mono-centre study, the TOI-based technique allowed for the individual analysis of predefined tract structures in the sample of 329 data sets from ALS-patients including longitudinal acquisitions. That way, *in vivo* stratification of the ALS-related disease patterns has become feasible, both cross-sectionally and longitudinally. This approach might enlarge the spectrum of potential non-invasive surrogate markers as a neuroimaging-based read-out for clinical trials in ALS.

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P71 PHENOTYPE-SPECIFIC WHITE MATTER SIGNATURES IN ALS

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Keywords: phenotype, biomarker, motor system

Background: A larger number of imaging studies propose MRI-based imaging measures as objective biomarkers of ALS. Magnetic resonance diffusivity indices are well-established markers of white matter integrity, but no consensus exists as to which diffusivity parameter is the most sensitive to identify early degenerative changes. While corticospinal tract degeneration is a hallmark feature of ALS, relatively little is known of its segmental vulnerability. Diffusivity changes in the corona radiata are surprisingly understudied compared to those in the posterior limb of the internal capsule.

Objectives: One of the objectives of this study was to characterise the core three-dimensional white matter signature of ALS and to describe phenotype-specific patterns of white matter degeneration. The main objective of the study was to anatomically segment the corticospinal tract and evaluate white matter diffusivity profile of individual patients and controls within these segments.

Methods: A large, single-platform, single-protocol, multimodal neuroimaging study has been undertaken with 26 patients with bulbar onset, 36 patients with spinal onset and 55 age-matched healthy controls. Tract-based white matter alterations were explored based on fractional anisotropy, radial-, mean-, and axial diffusivity indices. Atlas-based region of interest (ROI) analyses were carried out in the corona radiata, internal capsules, mesencephalic cruri, and corpus callosum.

Results: Bulbar and spinal onset patients demonstrated distinctive white matter changes in the corona radiata and internal capsule. The identified phenotype-specific patterns closely reflect the functional organisation of these tracts. Bulbar onset patients exhibited extensive corticobulbar tract involvement in the genu of the internal capsule and in the lateral fibres of the corona radiata subjacent to the bulbar representation of the motor homunculus. Spinal onset patients show predominantly posterior internal capsule involvement and medial corona radiata pathology. Individual patient data analysis revealed that diffusivity measures of the superior corona radiata best discriminate patients and controls, better than other segments of the corticospinal tract.

Discussion and conclusion: ALS is associated with a core, disease-specific 3D white matter signature, which is best demonstrated by radial diffusivity measurements. The main ALS motor-phenotypes are manifestations of the relatively selective involvement of corticospinal and corticobulbar fibres. Diffusivity values of the superior corona radiata reliably distinguish patients and controls better than the previously proposed internal capsule measurements. This study further supports the notion that diffusion tensor imaging is one of the most promising markers of ALS. The findings of this study have pragmatic implications for the development of automated classifier pipelines.

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P72 A THREE TIME POINT, LONGITUDINAL IMAGING ANALYSIS IN ALS

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Keywords: longitudinal progression biomarkers

Background: Past studies have attempted to characterise the longitudinal evolution of central imaging changes in ALS, often with contradictory results (1, 2). No previous studies have examined more than two-time points.

Objectives: To determine the utility of structural and diffusion imaging biomarkers compared to the standard clinical measure, ALSFRS-R (3), using three time-point measurements.

Methods: The group (N=34) comprised patients with classical ALS who had been scanned three times; first scan, mean age 57.39.9 years; mean time from symptom onset 24.221.0 months; two more scans at three months time intervals. ALSFRS-R severity score was assessed by the same experienced neurologist in all cases. The MRI protocol included structural MP-RAGE and diffusion tensor imaging (DTI) sequences as well as a T2 weighted sequence to exclude cases with vascular comorbidity. Cortical thickness of the precentral gyrus from the MP-RAGE and quantitative DTI biomarkers of the corticospinal tracts were assessed. MP-RAGE was also used to measure sub-cortical brain volumes and to perform voxel-based morphometry. The evolution of imaging and clinical biomarkers over time was analysed using a mixed effects model that accounted for the scanning interval as a fixed effect variable, and, the initial measurements and time from onset as random variables. The significance level for the longitudinal analysis was set to $p<0.05$.

Results: The mixed effects model showed a significant decrease in the severity score, (t=-7.87, p<1e-12, and an annual rate of change (AROC) of -7.3). Similarly FA also showed a significant decrease (t= -2.65, p=0.009, AROC=0.66). No significant change in cortical thickness of the precentral gyrus was found (t=-0.62,p=0.54, AROC =-0.016 mm). Subcortical volumes and voxel-based morphometry also identified no significant changes.

Discussion and conclusions: The results indicate that DTI is clearly a superior imaging marker compared to atrophy for tracking the evolution of the disease. It is, however, considerably less sensitive than the ALSFRS-R score for monitoring decline over time.

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P73 APPARENT DIFFUSION COEFFICIENTS DISTINGUISH AMYOTROPHIC LATERAL SCLEROSIS FROM CERVICAL SPONDYLOTIC MYELOPATHY

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Keywords: apparent diffusion coefficient, intracranial corticospinal tracts, cervical spondylotic myelopathy

Background: Fifty percent of patients with amyotrophic lateral sclerosis (ALS) have cervical spondylotic myelopathy (CSM) as a complication (1). Because most patients with ALS do not develop bulbar signs at onset (2), differentiating them from patients with CSM, especially those who do not show sensory symptoms (3) is sometimes difficult.

Objective: We aimed to determine whether the apparent diffusion coefficients (ADCs), which have emerged as useful quantitative index for detecting neurodegeneration (4), of intracranial corticospinal tracts can be used to distinguish between patients with ALS and those with CSM.

Methods: We evaluated 19 consecutive patients with ALS who did not have CSM by cervical MRI, 16 patients with CSM, and 11 healthy controls. We examined the mean ADCs in the precentral gyrus, the corona radiata, the posterior limbs of the internal capsule (PLIC), and the cerebral peduncle by 3 Tesla magnetic resonance imaging. The mean ADCs in the intracranial corticospinal tracts in patients with ALS were compared with those in patients with CSM.

Results: The mean ADCs in the intracranial corticospinal tracts in patients with ALS were compared with those in patients with CSM ($p < 0.05$). Additionally, the mean ADCs in the precentral gyrus, the PLIC, and the cerebral peduncle in the patients with ALS, including the patients who were initially diagnosed as having clinically possible ALS on the basis of the revised El Escorial criteria and did not develop bulbar symptoms at onset, were also higher than those in patients with CSM ($p < 0.05$).

Discussion and conclusions: Our study demonstrated significant elevations of ADCs in the intracranial corticospinal tracts in patients with ALS, but not in those with CSM. Note that even though patients with ALS initially showed only mild symptoms mainly with lower motor neuron signs and without bulbar signs, they showed significantly higher ADCs than patients with CSM. Elevated ADCs in the intracranial corticospinal tracts might be useful for distinguishing ALS from CSM in the early stage of the disease.

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P74 STRUCTURAL BRAIN MRI ABNORMALITIES IN KENNEDYS DISEASE

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Keywords: Kennedys disease, white matter, diffusion tensor MRI

Background: Kennedy's disease (KD) is a rare X-linked neurodegenerative disorder affecting the lower motor neurons, associated with a trinucleotide repeat expansion in the first exon of the androgen receptor (AR) gene. Preliminary studies suggest that KD might be more than a selective disorder of the lower motor neurons, highlighting the possible multisystemic nature of the disease. However, the extent of central nervous system involvement relative to amyotrophic lateral sclerosis (ALS) still needs to be clarified.

Objective: In this study, we investigated cortical and white matter (WM) alterations in a large sample of KD patients compared to healthy subjects and sporadic ALS patients.

Methods: 19 patients with genetically confirmed KD were compared with 21 age and sex matched healthy subjects, and 17 sporadic, non-demented ALS patients matched for demographics and ALSFRS-r score. All

patients underwent neurological examination, neuropsychological assessment, T1-weighted and diffusion tensor (DT) MRI. Tract-based spatial statistics was applied to investigate DT MRI metrics of WM tracts. Cortical thickness analysis was performed to identify cortical atrophy. ANOVA models were used to compare MRI features between groups.

Results: KD patients were characterized by pronounced behavioral symptoms and only subtle cognitive deficits. Relative to controls, KD patients showed decreased fractional anisotropy (FA) of the pontine crossing fibers, right frontotemporal and fronto-occipital tracts, and increased mean diffusivity (MD) and radial diffusivity of the right cingulum. They also showed a subtle cortical thinning of the inferior frontal gyrus bilaterally, left premotor regions and middle temporal gyrus, and right precuneus. ALS patients, compared to controls, showed the classic pattern of reduced FA and increased MD of the corticospinal tracts (CST) and corona radiata bilaterally, with an additional involvement of the left superior longitudinal, frontotemporal and fronto-occipital tracts, and cortical thinning of precentral gyrus, frontal cortex, lateral temporal and parietal regions, and precuneus bilaterally. The involvement of the CST, corpus callosum, external capsule bilaterally, and left SLF was greater in ALS compared with KD.

Discussion and conclusions: Our findings demonstrated subtle cortical abnormalities and the involvement of long-range frontal and limbic connections in patients with KD. Such changes may be related to the behavioural abnormalities of these patients. The pattern of damage of frontal and limbic WM tracts is similar in KD and ALS, while DT MRI measures of the CST and corpus callosum are proven to be powerful tools to differentiate ALS from mimic syndromes, including KD.

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P76 ELECTROMYOGRAPHIC FINDINGS OF PROGRESSIVE MUSCULAR ATROPHY: COMPARISON WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: electromyogram, progressive muscular atrophy, fasciculation potential

Background: Progressive muscular atrophy (PMA) is characterized by lower motor neuron (LMN) syndrome. With no upper motor neuron (UMN) signs, PMA does not meet the current diagnostic criteria of amyotrophic lateral sclerosis (ALS). However, a recent large-cohort study showed that PMA should be considered a form of ALS. Previously, there has been no sufficient consideration about the difference of EMG findings between PMA and ALS.

Objectives: To elucidate whether EMG findings of PMA are different from or the same as those of ALS.

Methods: We enrolled thirty-eight consecutive patients who underwent needle EMG for a diagnosis of motor neuron disease in our hospital from October 1, 2013 to June 30, 2014. The patients included seven PMA patients and 31 ALS patients with clinically definite or probable according to the Awaji criteria. We examined clinical ALS features (sex; age of onset; disease duration; ALSFRS-R scores at the time of EMG examination; forced vital capacity) and spontaneous EMG discharges (fibrillation potential; positive sharp wave; and fasciculation potentials) of five muscles (upper trapezius; biceps brachii; first dorsal interosseous; vastus medialis; and tibialis anterior muscles) to find any differences between PMA and ALS. For fasciculation potentials, we performed a quantitative analysis for their morphology.

Results: There were no significant difference in the clinical features between PMA and ALS patients. We also found no significant differences in EMG findings between PMA and ALS, both for the occurrence of denervation potentials and the morphology of fasciculation potentials.

Conclusion: PMA might share a common pathophysiology to ALS regarding not only the clinical disease courses but also the needle EMG findings.

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P77 POWER ANALYSIS OF ELECTROENCEPHALOGRAPHIC MU RHYTHM IN PATIENTS WITH ALS

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Keywords: EEG, mu rhythm, thalamus

Background: EEG mu rhythm is generated over the sensorimotor cortical regions. The thalamus acts as its pacemaker. There are few reports of the decrease of mu power and of thalamic neuron degeneration in ALS.

Objectives: Our aim was to evaluate longitudinal changes of mu rhythm in patients with ALS.

Methods: 17 patients were compared to healthy controls. EEG was recorded in supine position with eyes closed. 3 consecutive recordings (every 3 months) were done in 11 of the patients. Power analysis was performed and log transformed data were statistically analysed using repeated measures ANOVA. Structural MRI brain scans were acquired in 12 patients and in 12 healthy controls. Volumetric measurements of thalami were obtained from T1-weighted images.

Results: Mu rhythm power was significantly increased in patients compared to controls. ANOVA repeated measures with post hoc testing showed significant differences

between 1st and 3rd, and 2nd and 3rd EEG recording in patients, with an initial slight increase and then decrease of mu power over time. We observed significantly reduced thalami volumes in patients.

Discussion: Our findings could be interpreted as an interplay between changes of the cortical excitability, progressive deterioration of the pyramidal cells in the cortex and impairment of thalamo-cortical connections with disease progression.

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P78 EXPLORING MOTOR IMAGERY AND MOTOR CORTICAL FUNCTION IN AMYOTROPHIC LATERAL SCLEROSIS USING MAGNETOENCEPHALOGRAPHY

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Keywords: Magnetoencephalography, biomarker, motor imagery

Background: There is increasing evidence that cortical motor network dysfunction is a key pathogenic feature in ALS (1). Cortical neural networks underlying motor imagery overlaps with motor execution networks (2) and can be consistently activated even by patients with severe motor weakness. This offers an alternative approach to study motor network activity in ALS. Owing to its high temporal resolution, magnetoencephalography (MEG) is well-suited to localise oscillatory brain wave dysfunction during both executed and motor imagery tasks.

Objective: The aims of this pilot study are to determine (i) if ALS patients can perform a validated motor imagery task; and (ii) if MEG can be used to measure cortical motor responses from ALS patients longitudinally.

Methods: Spontaneous brain activity was recorded using a 160 channel whole-head KIT-Macquarie MEG in 4 ALS patients without dementia, and 4 healthy controls, during a novel hand motor imagery task (2). This novel imagery task has been shown to consistently activate the primary motor cortex during both executed and imagined tasks. ALS patients were clinically staged using the Amyotrophic Lateral Sclerosis Functional Rating Scale-revised (ALSFRS-R) and followed up longitudinally at 3 and 6 months.

Results: Both ALS patients and control participants achieved high levels of accuracy in the executed and imagined tasks (>90%). Beta band desynchronization, an index of motor cortex activity, was observed during both executed and imagined tasks, in both ALS patients and controls.

Discussion and conclusions: We have demonstrated in this pilot study that motor imagery task could be used to probe motor cortical function in ALS patients. This has a distinct advantage over traditional method of investigating cortical function using motor execution tasks, because it eliminates the bias associated with progressive muscle weakness. These results demonstrate that it is feasible to use magnetic source imaging and motor imagery in ALS to (i) identify early biomarkers of cortical involvement in ALS, and (ii) to examine longitudinal effects of progressive motor neuron degeneration on cortical motor network function in ALS patients.

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P79 SUB-THRESHOLD REPEAT LENGTH IN C9ORF72 CORRELATES WITH BRAIN-COMPUTER INTERFACE PERFORMANCE

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Keywords: brain-computer interface, electroencephalography, C9ORF72

Background: Brain-computer interfaces (BCIs) offer people with amyotrophic lateral sclerosis (ALS) alternative means of communication. The utility of these devices have been shown by our group to be negatively affected by cognitive impairment, which can be present in up to half of ALS patients (manuscript submitted). ALS and frontotemporal dementia (FTD) have a common genetic linkage in the pathological repeat expansion (30 or more) of hexanucleotide GGGGCC (G4C2) in the *C9ORF72* gene. The relationship between this genetic expansion, cognition, and BCI performance is unknown.

Objective: To measure the effect of the C*9ORF72* hexanucleotide expansion size on BCI utility in patients with ALS.

Methods: Twenty-four patients with ALS and fourteen control participants completed four sessions of electroencephalography-based P300 and motor-imagery BCI training. Each patient was also administered the ALS-Cognitive Behavioral Screen, and had repeat length

of *C9ORF72* analyzed. Wilcoxon rank sum tests were used to determine whether cognitive impairment, behavioral impairment, or site of onset were associated with significant differences in repeat length. Regression was used to determine correlations between repeat length and both age at symptom onset and BCI control signal quality.

Results: All patients in the study possessed G4C2 repeat lengths of fourteen or less, which is below the threshold known to be associated with ALS or FTD. Mean repeat lengths tended to be higher in participants who were cognitively or behaviorally impaired, although these trends were not significant. Patients with lower repeat lengths demonstrated consistently better quality in the P300 evoked potential, and there was a negative correlation between repeat length and P300 quality ($R^2 = 0.21$, $p = 0.024$). For the motor-imagery task, high performers displayed elevated signal quality over the 5-30 Hz range typical of a motor-imagery task. Averaged over this frequency range, motor-imagery quality was negatively correlated with repeat expansion length across patients ($R^2 = 0.19$, $p = 0.033$).

Discussion and conclusions: We have shown that longer repeat expansions of *C9ORF72*, even when below the threshold associated with ALS and FTD, are associated with poorer BCI performance. These results raise the possibility of a genetic screen for BCI utility, as well as the development of BCI as a diagnostic tool for early detection of non-motor manifestations of ALS. These findings warrant further exploration of the effects of repeat expansion on spontaneous and evoked brain phenomena, the relationship of sub-threshold repeat expansions and cognitive decline, and the utility of BCI as a communication tool in ALS patients with varying levels of cognitive function.

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P80 USING ELECTRICAL IMPEDANCE MYOGRAPHY TO PREDICT FORCE OUTPUT IN ALS: A STUDY IN THE G93A SOD1 MOUSE

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Keywords: impedance, force, strength

Background: Tools to rapidly and quantitatively predict force output of muscle based on muscle at rest could serve as valuable biomarkers in clinical trials in ALS. Previous work in both ALS patients and animal models has shown that electrical impedance myography (EIM) correlates to clinical measures of force, including handheld dynamometry in humans and paw grip endurance in mice. However, the more precise relationship between impedance and true force generating capability is unknown.

Objectives: We evaluated the relationship between single- and multi-frequency EIM parameters and both isometric and isotonic force production in the ALS mouse.

Methods: Twenty-eight ALS (B6SJL-Tg(SOD1-G93A)1Gur/J) mice approximately evenly distributed from 13 to 18 weeks of age were separated to two groups based on standard clinical criteria: an asymptomatic group, with no evidence of motor dysfunction; a symptomatic group, with clinical signs, including hind limb tremors or weakness. Standard front/hind paw grip strength were assessed with the animals awake. Multi-frequency EIM measurements were then performed on the gastrocnemius muscle under anesthesia; measures to be evaluated included the 50 kHz phase and resistance values, and the center frequency, f_c, a multi-frequency measure of the peak reactance. Maximum isometric force (P_{max}) and maximum contraction velocity (V_{max}) were then performed on the exposed gastrocnemius muscle using an Aurora Scientific muscle force measurement system. Mann-Whitney tests were used for two-group comparisons and non-parametric (Spearman) tests were performed for all correlations.

Results: The symptomatic group had reduced strength in front ($p<0.001$) and hind limbs ($p<0.001$). These animals had lower P_{max} ($p<0.002$) and slower V_{max} ($p<0.01$). These differences were reflected in the EIM data, with the symptomatic animals having lower 50 kHz phase ($p<0.001$), higher 50 kHz resistance ($p<0.01$), and higher f_c ($p<0.001$). Strong correlations between EIM and force production were similarly identified across all the animals. Phase, resistance, and f_c correlated to front paw grip strength ($r=0.74$ ($p<0.001$), -0.58 ($p<0.001$), and -0.78 ($p<0.001$), respectively) and to hind paw grip strength ($r=0.72$ ($p<0.001$), -0.40 ($p<0.05$), and -0.84 ($p<0.001$), respectively). Phase, resistance, and f_c also correlated to P_{max} ($r=0.48$ ($p<0.02$), -0.49 ($p<0.02$), and -0.78 ($p<0.001$), respectively); to V_{max} ($r=0.57$ ($p<0.01$), -0.54 ($p<0.02$), and -0.84 ($p<0.001$), respectively).

Discussion and conclusions: This study reveals that both isometric and isotonic force production in the ALS mouse decreases with progressive disease and that corresponding EIM changes are present. In addition, the heretofore unstudied multifrequency impedance parameter, the center frequency, which is strongly related to myocyte size, appears to be even more correlated to the functional measures than standard 50 kHz parameters. Additional study and application of these impedance measures in ALS animals and human studies is warranted.

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P81 THE NEEDLE ELECTROMYOGRAPHY FEATURES IN 112 PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: electromyography, spontaneous activity, forced vital capacity (FVC)

Background: Amyotrophic lateral sclerosis (ALS) is marked by ongoing motor unit loss. Hence, the needle electromyography can detect signs of lower motor neuron loss, which may also be used to stratify the ALS patient population and potentially identify sub-populations that may exacerbate rapidly.

Objective: To analyze the features of needle electromyography in patients with ALS, and explore the correlation between EMG parameters of lower thoracic paraspinal muscle and disease duration, rate of disease progression, forced vital capacity (FVC) and revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R).

Methods: Standard EMG was recorded from unilateral sternocleidomastoid muscle and/or tongue muscles, upper and lower limbs muscles and lower thoracic paraspinal muscle in 112 patients with definite ALS. Neurophysiological tests included spontaneous potentials, duration and amplitude of motor unit potentials (MUP), pattern of recruitment.

Results: EMG revealed diffuse neurogenic changes in each case. Fibrillation potentials or positive sharp waves were found in some muscles in the ALS patients, and both of them were found in most of the cases. Fasciculation potentials (FPs) were found in 8 patients and accompanied with fibrillation potential and positive sharp wave potentials in 5 of them. Complex repetitive discharges (CRD) were found in only 1 patient. Long duration and high amplitude MUP were detected in each ALS patient, and the rate of incomplete interference pattern and high amplitude, decreased recruitment pattern were 72.32% and 35.71%, respectively. Logistic regression analysis revealed a relation of the FVC loss with the drop of ALSFRS-R and peak MUP amplitude of lower thoracic paraspinal muscle (OR=1.157; OR=2.363).

Discussion: The present study indicates that fibrillation potentials and/or positive sharp waves were the most common spontaneous potentials in ALS patients. In distal limbs the fibrillation potentials and positive sharp waves that appear more frequently. Meanwhile, our data showed low occurrence frequency of FPs which greatly differ from other studies. It is suggested that there is a racial and population difference in ALS. Logistic regression suggests a relation of the FVC loss with the drop of ALSFRS-R and peak MUP amplitude of lower thoracic paraspinal muscle, which provide evidence for the disease progression of motor neuron loss in ALS. Furthermore, the peak MUP amplitude of lower thoracic paraspinal muscle is objective, informative, easy to interpret and appears to represent disease progression more sensitively and accurately than ALSFRS-R in ALS patients with respiratory dysfunction.

Conclusion: The most common abnormal spontaneous activity were fibrillation potentials and/or positive sharp waves. However, FPs were observed in a small number of patients. The ALSFRS-R and peak MUP amplitude of lower thoracic paraspinal muscle may be of certain clinical value in estimating the severity of disease especially the respiratory dysfunction in ALS patients.

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P82 EVALUATION OF MUNIX MEASUREMENTS IN ALS PATIENTS AS CLINICAL ROUTINE PROCEDURE IN A SPECIALIZED NEUROMUSCULAR TREATMENT UNIT

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Keywords: MUNIX, neuromuscular decline, biomarker

Background: Different motor unit number estimation methods were suggested as surrogate markers to quantify the underlying decline of motor units in ALS and other neurodegenerative diseases. However, these methods were time-consuming and did not find their way into clinical routine. Recently, motor unit number index (MUNIX) was introduced as a fast new technique, relying on surface interference patterns (SIP) recorded during voluntary contractions. MUNIX values decrease with disease progression comparable to the ALS functional rating score in a lab environment where interrater variability was reduced by intense rater training.

Objective: Our goal was to implement MUNIX as routine procedure in a specialized neuromuscular unit in realistic medical care conditions, to determine variability and benefit in clinical routine in ALS patients.

Methods: MUNIX-measurements were performed prospectively in seventy ALS patients cross-sectionally in up to four muscles (APB, ADM, TA, EDB) by trained raters. Quality scores were obtained to compare reliability between different measurements and interrater-variability.

Results: MUNIX values were significantly reduced in ALS patients in different muscles (meanSD: APB 52.848.2; ADM 62.141.3; EDB 38.236.1; TA 69.846.5) versus controls (APB 17843.9; ADM 12733.7; EDB 75.140.6; TA 11639.4) and showed a marked variability. Quality index was negatively correlated with the compound motor unit potential (CMAP) amplitude and MUNIX values, leading to poorer measurement quality in small CMAP and MUNIX values. Despite extensive application in a clinical routine setting, quality markers did not improve over several months.

Conclusion: MUNIX measurements in clinical routine show marked variability, which may not be eliminated completely by intense rater training. We assume at this stage that the source of this variability is patient associated rather than rater dependent because the same amount of outliers were detected during a period of > 6 months. It is now necessary to identify in which patients the variability was highest.

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P83 INCREASING WEAKNESS: DOES MRI SHOW ANYTHING? LESSONS FROM SINGLE SUBJECT ANALYSIS

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Keywords: DTI, TRACULA, cortical thickness

Background: We share our experience imaging a left-handed patient with ALS exhibiting progressive left > right upper extremity weakness. In doing so, we compare automated probabilistic tractography with deterministic approaches.

Methods: Initial and 1-year follow-up 3T MRI scans included 3D T1 MPRAGE and 64-direction DTI. Cortical reconstruction and volumetric segmentation was performed with Freesurfer and average primary motor cortex (PMC) thickness was measured. Tailored deterministic tractography was performed first by a CAQ neuroradiologist; subsequent tract statistics were collected. Automated probablistic tractography (yielding statistics) was then performed with TRACULA.

Results: Preliminary results demonstrate asymmetries in PMC thickness and CST FA. Right PMC thickness decreased (2.062à1.862mm) while left (2.099à2.141mm) was similar. FA decreased and RD increased in both CSTs over time, with both DTI approaches.

Probabilistic CST FA: right 0.574à0.526; left 0.599à0.506

Deterministic CST FA: right 0.612à0.516; left 0.642à0.542

Conclusion: Preliminary analysis reveals differences and changes in SMC cortical thickness and CSTs that may reflect clinical status. While general trends in DTI results are similar, subtle differences illustrate the impact of different post-processing approaches. We anticipate examining additional patients, SWI, additional white matter tracts, and identification of a control tract, to optimize single-subject characterizations.

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P84 THE SPLIT-HAND SIGN IN AMYOTROPHIC LATERAL SCLEROSIS: DIFFERENT F-WAVE CHARACTERISTICS BETWEEN THE MEDIAN AND ULNAR NERVES

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Keywords: split-hand sign, F-wave, motor neuron

Background: In amyotrophic lateral sclerosis (ALS), hand muscle wasting preferentially affects the abductor pollicis brevis (APB) and first dorsal interosseous muscles, with relative preservation of the abductor digiti minimi (ADM). This peculiar pattern of dissociated hand muscle atrophy is termed the split-hand sign.

Objectives: F-wave allows the non-invasive assessment of spinal motoneuron excitability. This study aimed to determine whether differences in spinal motoneuron dysfunction between the APB and the ADM follow a split-hand pattern in ALS patients based on F-wave characteristics.

Methods: F-waves of the APB and the ADM were compared between 40 ALS patients and 20 normal controls.

Results: The F-wave persistence ($P = 0.002$); Index repeating neuron (RN) ($P < 0.001$); Index repeater F-waves (Freps) ($P < 0.001$); and the F-wave conduction velocity ($P = 0.022$) significantly differed between the APB and the ADM in the control subjects. In ALS patients with hands lacking detectable wasting or weakness, significantly reduced F-wave persistence ($P < 0.001$); increased Index RN ($P < 0.001$); and increased Index Freps ($P < 0.001$) of the APB were observed, compared to the relatively normal F-waves of the ADM. In ALS patients with hands exhibiting wasting and weakness, the mean F-wave amplitude ($P = 0.001$); the F/M amplitude ratio ($P = 0.012$); the F-wave persistence ($P < 0.001$); Index RN ($P = 0.038$); and Index Freps ($P = 0.018$) significantly differed between the APB and the ADM.

Conclusions: F-waves followed the split-hand pattern in both the normal subjects and the ALS patients. These findings likely reflected the specific pathological mechanisms underlying ALS.

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P85 SYMPATHETIC SKIN RESPONSE IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: sympathetic skin response (SSR), autonomic nervous system

Background: Traditonally ALS has been considered to only involve motor neurons. However, increasing evidence from clinical, pathological and genetic studies has characterized ALS as a multi-system degeneration on contimuum with cognitive function and disorders with autonomic nervous system. The present study was carried out to investigate if there was involvement of the sympathetic skin response (SSR) in ALS.

Method: We investigated 120 patients with sporadic ALS and in one hundred thirty age-matched healthy subjects to determine the effects of SSR in ALS patients. The SSR was conducted in all ALS patients and healthy subjects by Dantec Keypoint EMG in a quiet and warm room.

Results: The mean SSR latency in the ALS patients was prolonged, the mean SSR amplitude was reduced compared to that of control subjects, especially in their low extremities ($P<0.05$). Duration of disease seemed to have little impact on the SSR latency and amplitude ($P>0.05$). Yet whatever arms or legs were involved, the overall difference in lower extremity SSR measurable between the two groups was considerable ($P<0.05$). There were no correlation during clinical manifestation of autonomic nervous impairment and SSR amplitude and latency ($P<0.05$).

Conclusion: The SSR impairment happens earlier than the clinical manifestation of autonomic nervous impairment in ALS. This maybe a sub-clinical manifestation of ALS. We hypothesise that these results are caused by damage to the non-myelinated postganglionic fibers in ALS patients.

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P86 S6K-MEDIATED CELLULAR PROPERTIES CHANGE IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: S6K, size, electrophysiology

Background: In ALS, spinal motor neurons with various sizes show distinctive degenerating rates in which large motor neurons are particularly vulnerable. ALS spinal motor neuron size is increased from neonatal stage accompanied with abnormal electrical properties. Motor neuron size enlargement may be the subsequence of initial responses to morbific prodromal factors such as altered expression level of proteins related to survival and translation. We hypothesize that the early-stage abnormal soma size and electrical properties might link to the selective susceptibility of motor neurons in ALS. S6K is selected for motor neuron size manipulation due to its regulatory roles in protein translation, cell growth and synapse development.

Objectives: Examine the motor neuron cell size and electrical properties after S6K inhibitor injection at neonatal stage. Assess the effects of S6K-suppression strategy on adult ALS mice by measuring motor function, disease progression and lifespan.

Methods: Lumbar spinal motor neuron size was examined by two-photon laser scanning followed by 3-D reconstruction and calculation. Electrical properties were determined by slice patch-clamp on lumbar spinal motor neurons. Behavioural assays were formed by measuring body weight, Rota-Rod performance and neurological symptoms. Data were showed as meanSEM; ANOVA and student t-test were performed, only $P < 0.05$ was considered significant.

Results: Our results confirmed that neonatal motor neuron size was abnormally enlarged ($P<0.05$) and that S6K inhibitor could maintain the size of lumbar spinal motor neurons from early stage G93A mice ($P<0.05$). Post-symptomatic S6K inhibitor administration onto adult mice revealed distinctive responses upon different doses. Lower dose mildly prolonged the lifespan (1318.07 vs. 140.257.93 days, $P=0.06$) but significantly improved motor function ($P < 0.05$), while the higher does specifically accelerated the disease progression (118.25.93, $P < 0.05$).

Discussion and conclusion: The early stage abnormality can be amended by S6K suppression which implies the early involvement of S6K pathway in ALS pathogenesis. Manipulating S6K activities showed dramatic impacts on disease progression and motor function, which demonstrates the therapeutic potential of S6K pathway. The distinctive dose-dependent responses suggested the importance of S6K management in ALS.

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Theme 5 Cognitive Change

P87 STRUCTURAL BRAIN CORRELATES OF COGNITIVE AND BEHAVIOURAL IMPAIRMENT IN MND

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Keywords: cognitive impairment, behavioural changes, diffusion tensor MRI

Background: There is increasing evidence that amyotrophic lateral sclerosis (ALS) is a clinically heterogeneous disease in terms of site of onset, degree of upper and lower motor neurons involvement, and rate of motor progression. Several studies indicate that this heterogeneity also includes the presence and severity of cognitive and behavioral symptoms.

Objective: To assess the structural correlates of cognitive and behavioral impairment in motor neuron disease (MND) using multimodal magnetic resonance imaging (MRI).

Methods: 101 patients with sporadic MND and 51 controls were enrolled in this study. Patients were classified into MND with a pure motor syndrome (MND-motor) and with cognitive/behavioural symptoms (MND-plus) based on a comprehensive neuropsychological evaluation. Cortical thickness measures and diffusion tensor (DT) metrics of white matter (WM) tracts were assessed. A random forest approach was used to explore the independent role of cortical and WM abnormalities in explaining major cognitive and behavioral symptoms.

Results: There were 48 MND-motor and 53 MND-plus patients. Relative to controls, both patient groups showed a distributed cortical thinning of the bilateral precentral gyrus, insular and cingulate cortices, and frontotemporal regions. In all regions, there was a trend towards a more severe involvement in MND-plus cases, particularly in the temporal lobes. Both patient groups showed damage to the motor callosal fibers, which was more severe in MND-plus. MND-plus patients also showed a more severe involvement of the extra-motor WM tracts. The best predictors of executive and non-executive deficits and behavioral symptoms in MND were diffusivity abnormalities of the corpus callosum and frontotemporal tracts, including the uncinate, cingulum, and superior longitudinal fasciculi.

Discussion and conclusions: Cortical thinning and WM degeneration are strongly associated with neuropsychological and behavioral symptoms in patients with MND. DT MRI metrics seem to be the most sensitive markers of extra-motor deficits within the MND spectrum. The longitudinal assessment of our MND patients is ongoing and would likely identify MRI features that can have a predictive value in this population.

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P88 CORTICAL ATROPHY CORRELATES OF COGNITIVE AND BEHAVIORAL SYMPTOMS ACROSS THE ALS-FTD CONTINUUM

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Keywords: Amyotrophic Lateral Sclerosis-Frontotemporal Dementia (ALS-FTD), cortical atrophy, cognitive/behavioral symptoms

Background: Amyotrophic Lateral Sclerosis-Frontotemporal Dementia (ALS-FTD) may present with symptoms typical of behavioral variant FTD (bvFTD) such as apathy, disinhibition and/or executive dysfunction. Focal lesion studies suggest an association between these symptoms and specific frontal regions (1). Although cognitive and/or behavioral symptoms in ALS are widely reported, there is very little literature on the relationship between regional gray matter atrophy and specific cognitive/behavioral symptoms. In ALS, apathy has been associated with atrophy in orbitofrontal and dorsolateral prefrontal cortices, while impaired action knowledge

correlated with atrophy in motor cortex and anterior temporal lobe atrophy with semantic deficits (voxel-based morphometry studies). To date, there are no evaluations identifying associations between specific cognitive or behavioral symptoms in ALS-FTD and specific regional reduced cortical thickness.

Objectives: To evaluate the hypothesis that the types and severity of cognitive and behavioral symptoms in ALS/ALS-FTD would be predicted by regional cortical atrophy. Specifically, we hypothesize that the severity of executive dysfunction would be predicted by dorsolateral prefrontal cortex (dlPFC) atrophy, apathy by dorsomedial PFC (dmPFC) and anterior cingulate (ACC) atrophy, and disinhibition by orbitofrontal (OFC) atrophy.

Methods: 3.0 Tesla MRI scans were acquired from 114 controls, 10 ALS-FTD and 12 ALS patients. Quantitative cortical thickness analysis was performed with Freesurfer. A priori-defined, region of interest (ROI) were used to measure cortical thickness in each patient and calculate the magnitude of atrophy relative to controls. Spearman correlations were used to evaluate associations between frontal ROIs and behavioral symptoms of interest, measured by Neuropsychiatric Inventory Questionnaire (NPI-Q) and Clinical Dementia Rating (CDR) scale.

Results: ALS patients did not exhibit any cognitive or behavioral symptoms (by definition), while ALS-FTD subjects showed variable degree of apathy (NPI-Q/apathy: 1.61.2), disinhibition (NPI-Q/disinhibition: 1.21.2), executive dysfunction (CDR/judgment-problem solving: 1.70.8). In ALS-FTD patients, executive dysfunction correlated with dlPFC atrophy (ρ:-0.647;$p<0.05$); similar trends were seen for apathy with ACC atrophy (ρ:-0.532;$p<0.10$) and disinhibition with OFC atrophy (ρ:-0.505;$p<0.10$). Compared to ALS, ALS-FTD showed more diffuse atrophy involving the precentral gyrus, prefrontal and temporal regions.

Discussion and conclusions: The concomitant absence of both behavioral symptoms and cortical atrophy in the putative regions of interest in the ALS population supports the behavioral-anatomical correlations seen in ALS-FTD cases. As predicted, the types and severity of cognitive and behavioral symptoms in ALS-FTD were predicted by regional prefrontal atrophy, supporting concepts of brain-behavior relationships derived from studies of patients with focal brain lesions.

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P89 18F-FDG-PET CORRELATES OF COGNITIVE IMPAIRMENT IN ALS

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Keywords: cognitive impairment, 18F-FDG-PET

Background: Overall, ~15% of ALS patients display a full-blown frontotemporal dementia (FTD), while 35% have more subtle cognitive alterations involving executive and non-executive domains. It is still unclear whether the clinical entities of such disease spectrum have distinct metabolic correlates reflecting the different degrees of cognitive impairment.

Objectives: To identify the metabolic signature of the various levels of cognitive deficits in ALS using 18F-FDG-PET.

Methods: A total of 170 ALS cases consecutively enrolled at the ALS Center of Turin underwent brain 18F-FDG-PET and were classified as displaying normal cognition (ALS-Cn=94); full-blown FTD (ALS-FTD=20); executive or non-executive cognitive impairment not fulfilling FTD criteria (ALS-Ci=37); prevalent behavioral changes (ALS-Bi=9); or non-classifiable impairment (ALS-Nc=10) according to neuropsychological testing. Group comparisons of 18F-FDG-PET pattern were carried out among the cognitive subgroups.

Results: We found a significantly reduced frontal and prefrontal metabolism in ALS-FTD as compared to ALS-Cn, while ALS-Ci showed an intermediate metabolic behavior in frontal cortex, being hypometabolic as compared to ALS-Cn, and relatively hypermetabolic as compared to ALS-FTD. Hypometabolism in frontal regions was associated in all comparisons to hypermetabolism in cerebellum, midbrain and corticospinal tracts: the more severe the cognitive decline, the larger the size of the cluster and the statistical significance of 18F-FDG uptake differences.

Discussion: This is the first study demonstrating in a large ALS cohort, a continuum of frontal lobe metabolic impairment reflecting the clinical and anatomic continuum ranging from pure ALS, through ALS with intermediate cognitive deficits, to ALS-FTD, and showing that patients with intermediate cognitive impairment display a characteristic metabolic pattern. Since 18F-FDG-PEt allows the estimation of the cerebral lesion load *in vivo* in neurodegenerative diseases, it might be helpful to investigate the neurobiological basis of cognitive impairment in ALS along the disease course, showing the early regional spreading of brain metabolic alterations.

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P90 ASSOCIATION OF OXIDATIVE STRESS BIOMARKERS AND COGNITIVE EVENT RELATED POTENTIALS IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: oxidative stress, event related potentials, cognitive impairments

Background: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder recognized to include the mechanisms of oxidative stress (OS). Due to the increase of reactive oxygen species (ROS), the OS is defined as a disequilibrium of redox-dependent signalling pathways (1). This phenomenon has been associated to neurodegenerative mechanisms and some clinical and genetic features in ALS (2). In recent years, cognitive deficit has been demonstrated in this neurological disease (3). However, there is no evidence of a possible association between cognitive dysfunction and oxidative damage in ALS patients.

Objective: To determine the relationship between oxidative stress and cognitive function disorders in ALS patients.

Methods: 10 ALS patients diagnosed according to the revised El Escorial Criteria were studied. Oxidative damage was analyzed in blood by measuring malondialdehyde (MDA) and advanced oxidative proteins products (AOPP). Superoxide dismutase (SOD1) activities, catalase, SOD1/CAT ratio, glutathione peroxidase, protein thiols and total antioxidant capacity were determined. Event Related Potentials (ERPs) were recorded using 32 EEG channels montage. Electrical sources were estimated by means of Bayesian Model Averaging (BMA). Clinical variables, depression and cognitive status were also evaluated with standardized scales for ALS patients.

Results: Decreased P300 amplitude was statistically significant with MDA marker in parietal derivation. The higher the concentration of MDA; the smaller the P300 amplitude. GPx and SOD1/CAT ratio were directly associated with decreased amplitude in the N100 component in frontal electrodes. A significant correlation (R=0.90, p=0.03) between N100 amplitude and AOPP was obtained in frontal topography. P3 amplitude correlated with time of disease evolution and Beck depression inventory. Lower activation was found at the level of source generators of both hemispheres (frontal and temporal lobes) in ALS patients. No significant correlation was found between electrophysiological variables and other oxidative stress biomarkers.

Discussion and conclusion: These findings demonstrate that oxidative stress abnormalities are associated with cognitive deficits. Decreased N100 and P3 amplitude and increment of oxidative damage suggest the relation between ROS and the presence of sub-clinical cognitive deficits in ALS patients. Moreover, antioxidant status could be a defence against oxidative damage which has shown similar results in other diseases. Neuroimaging studies evidence the frontal and parietal lobes dysfunction reflecting cortical degeneration in ALS that could be associated to mechanisms of oxidative stress. The electrophysiological abnormalities found in brain areas could be caused by increased ROS.

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P91 ASSESSMENT OF COGNITIVE CHANGE FOR A PERSON WITH ALS/MND WITHIN THE CLINIC

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Keywords: cognitive change, assessment, ECAS

Objectives: An ongoing study is looking to see if cognitive change for a person with ALS/MND can be detected within the normal clinic setting, using the Edinburgh Cognitive and Behavioural ALS Screen (1) and a short B word test.

Methods: Patients have been assessed during an extended outpatient clinic appointment, at home or in the day hospice. Patients who had severe problems communicating, were anxious about their care and deterioration or were unwilling to be involved were excluded. The Edinburgh Cognitive Assessment Scale and a short B word test, asking the person to say as many words beginning with B in one minute, were used.

Results: Ten patients have been assessed: 30% were male; the mean age was 62 years; the mean time from diagnosis to testing was 45 months (range 17-71 months); and the mean ALSFRS-R score was 28. The ECAS was easy to administer and took 20-30 minutes and was possible to use when the patient used an iPad for communication.

Four patients had results below the cut-off level for cognitive change, of which only one had been considered as having FTD beforehand. The short B word test identified three of the four people with an abnormal

ECAS, and only identified one abnormal score in the group with a normal ECAS score.

Conclusions: The ECAS was easy to administer within a clinic or home setting and took on average 20 to 25 minutes. The use of a short B word test may be able to be used as a short screening test to identify people for whom the longer ECAS assessment would then be helpful.

The knowledge of the possibility of cognitive change has enabled the team to be more aware of these issues and to be proactive in the discussion of issues of care including advance care planning. It has also allowed the team to share the results with carers and other professionals involved in the persons care to be aware of possible issues in decision making and help them cope with the deterioration of the patients condition.

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P92 LONGITUDINAL ASSESSMENT OF THE EDINBURGH COGNITIVE ASSESSMENT (ECAS) - IS THERE A LEARNING EFFECT IN ALS PATIENTS?

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Keywords: ECAS, learning, cognitive changes

Background: Cognitive deficits and behavioral changes are well recognized symptoms in ALS. The Edinburgh cognitive and behavioral ALS screen (ECAS) (1, 2) has been recently validated in several languages as a fast and easy to administer cognitive screening tool for clinical routine. However, it is unclear how ALS patients perform, if the testing is applied serially.

Objective: To assess if controls and/or patients show a learning effect over time in the ECAS and if patients show a progression of cognitive or behavioral changes.

Methods: A previously validated Swiss-German version of the ECAS was administered serially in 40 ALS patients and 49 controls. In addition, the frontal assessment battery (FAB) was administered and carer behavior screens were performed.

Results: Twenty-four ALS patients and healthy controls were available for a second ECAS testing. Mean time between the two measurements was 205 days in controls and 195 days in ALS patients. In 15 controls and 17 ALS patients, the FAB could be repeated after 6 months. Nine ALS patients performed the ECAS and 5 the FAB for a third time after more than one year. Sixteen carer behavior screens were performed in second and 4 in third testing. Administration of ECAS was complete in all ALS patients at each measurement. The FAB could not completely be applied in several patients after 6 months and more than one year due to motor deficits. Controls showed a significantly higher overall score ($p<0.001$) and significantly higher score in several subdomains (ALS-specific $p<0.005$; ALS-non-specific $p<0.017$; memory $p<0.02$; language $p<0.025$; executive functions $p<0.01$) except for visuospatial function and fluency after 6 months. ALS patients showed no significant difference in overall scores or any subdomains after 6 months and after one year. The FAB could not detect any significant differences between controls and ALS patients. Behavioral changes were increasingly described in the carer behaviour interviews.

Discussion and conclusions: The Swiss-German version of the ECAS is a fast and easy to administer cognitive screening instrument and even applicable in later disease stages of ALS. Controls show a significant learning effect in a second testing after 6 months in most domains. By contrast, ALS patients seem to have no learning effect but present with progressive behavioral symptoms during the course of the disease.

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P93 EVALUATION OF THE EDINBURGH COGNITIVE AND BEHAVIORAL ALS SCREEN (ECAS) IN A US SAMPLE

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Keywords: frontotemporal dementia, cognitive testing, behavioral testing

Background: Up to 50% of patients with ALS exhibit cognitive impairment (1). Comprehensive neuropsychological testing is impractical in a multidisciplinary ALS clinic. The Edinburgh Cognitive and Behavioral ALS Screen (ECAS) is a brief screen that includes an ALS-Specific Score (Language, Verbal Fluency, and Executive domains), an ALS Non-Specific Score (Memory and Visuospatial domains), and a Behavioral Screen completed by a caregiver (2). It has been validated in the UK, demonstrating high sensitivity (85%) and specificity (85%) for cognitive dysfunction (3).

Objectives: 1) Assess the feasibility of performing the ECAS as a screen in a multidisciplinary ALS clinic; 2) Compare ECAS results from a US ALS clinic to UK data.

Methods: This is a retrospective review of data collected using an American (US) version of the ECAS administered in a multidisciplinary ALS clinic during routine clinical care. Exclusion criteria included: fatigue and/or weakness (including end-stage disease) precluding testing; severe pre-existing neurological or mental health conditions; insufficient clinic time. Testing was administered by certified clinic personnel. Descriptive statistics were compared to published UK data.

Results: Data was available on 53 patients (38 men, 15 women; aged 33 to 82 (*M*=59.9, *SD*=10.2)) and 51 caregivers (13 men, 38 women). Mean duration of disease was 34.6 months (*SD*=27.7, range=8-166 months). Mean ALSFRS-R Score was 33.5 out of a possible 48 (SD=6.9, range=16-43). ECAS administrations required 15-30 minutes (M20). Behavioral screening of caregivers required 5-20 minutes (M10). Abnormal ALS-nonspecific cognitive scores were found in 23 patients (43% vs. UK 29%) and abnormal ALS non-specific cognitive scores in 7 patients (14% vs. UK 6%). Deficits were found within language (59% vs. UK 35%), verbal fluency (43% vs. UK 23%), and executive (30% vs. UK 23%). 49% (vs. UK 40%) of caregivers reported changes in 1 behavioral domain, with 16% (vs. UK 15%) meeting ECAS criteria for possible frontotemporal dementia.

Discussion and conclusions: The ECAS can be administered in a multidisciplinary ALS clinic by individuals undergoing relatively brief training. Cognitive abnormalities were found more frequently in the US clinic sample than in the published UK data, although the relative frequency of ALS-Specific abnormalities was similar. The higher frequency in the US group may be due to different patient populations (clinic population *vs.* a registry population, different inclusion/exclusion criteria). Behavioral changes were similar in the US and UK groups. Further studies are warranted to establish true US normative data.

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P94 DEVELOPMENT OF PARALLEL VERSIONS OF THE EDINBURGH COGNITIVE AND BEHAVIOURAL ALS SCREEN (ECAS)

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Keywords: cognitive and behavioural assessment, screening, serial testing

Background: It is now well established that up to 50% of patients with ALS will experience difficulties in cognition and behaviour, particularly impairments in executive function, social cognition and language. It has been shown that cognitive impairment is a negative prognostic indicator, reinforcing the need for timely and appropriate assessment. The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) was developed for use with patients with ALS, while controlling for motor impairment. The ECAS provides a measure of executive functioning, language and verbal fluency (ALS-specific domains), as well as memory and visuospatial function (ALS non-specific domains). However, due to practice effects, common in cognitive testing, serial administration may mask cognitive decline.

Objectives: To develop two parallel versions of the ECAS to overcome potential practice effects by repeated administration.

Methods: Two additional versions of the ECAS were developed and administered to two groups of healthy adults (n=62) matched by age, gender and education to the original ECAS validation study (n=40) (1).

Results: Descriptive statistics indicate that mean scores obtained across the three ECAS versions for all subtests were within 1 point of each other. Results were analysed using Welch F-Ratio, Kolmogorov-Simonov, and Bayesian ANOVA to determine equivalency. Due to ceiling effects on some of the ECAS subtests, only the amalgamated ALS specific, ALS non-specific and ECAS total were subjected to further analysis. Welch F-ratio tests for ALS specific, ALS non-specific, and ECAS total were non-significant (p=0.700; p=0.720; p=0.625, respectively). Kolmogorov-Smirnov tests were also non-significant on comparison of version 1 to 2 (p=0.985; p=0.072; p=0.800) and of version 1 to 3 (p = 0.796; p = 0.635; p = 0.635). Finally, Bayesian ANOVAs favour the null hypothesis (BF_{01} = 8.04, 8.34, 7.41) over the alternative hypothesis (BF_{10} = 0.124, 0.120, 0.135).

Discussion and conclusions: Descriptive statistics suggest that expected scores across all three versions, and for all subscales of the ECAS, are similar. Results of the Welch F-ratio and the Kolmogorov-Smirnov tests

demonstrate that the observed means and distributions of scores are not significantly different between groups. The Bayesian ANOVAs provide substantial evidence in favour of the null hypothesis (that the scores come from the same distribution) over the alternative hypothesis. These findings reveal strong evidence for the equivalence of the parallel versions of the ECAS supporting their interchangeable use within clinical and research settings.

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P95 COGNITIVE CONTINUUM ACROSS AMYOTROPHIC LATERAL SCLEROSIS (ALS) AND FRONTOTEMPORAL DEMENTIA (FTD): AN INVESTIGATION ON SOCIAL COGNITION

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Keywords: frontotemporal dementia, social cognition, neuropsychology

Background: Besides genetic and pathological mechanisms, amyotrophic lateral sclerosis (ALS) and the behavioral-variant frontotemporal dementia (bvFTD) share common neuropsychological features. Executive and working-memory deficits are observed in both ALS and FTD patients. There is increasing evidence that functions related to social cognition, like theory of mind and emotional processing, are impaired in ALS to the same degree of bvFTD.

Objective: To investigate social cognition in ALS patients and to compare them with patients with bvFTD.

Methods: Forty-one participants were enrolled in this study, including fifteen ALS patients (mean age = 53 years-old; M/F, 8/7), eleven bvFTD patients (mean age = 58 years-old; M/F, 8/3) and fifteen healthy controls (mean age = 59 years-old; M/F, 10/5). We included ALS patients with probable or definite diagnosis according to Awaji's criteria, and patients with probable bvFTD according to Rascovsky's criteria. All participants underwent standardized clinical, neuroimaging (brain MRI) and neuropsychological assessments. Cognitive evaluation focused on executive functions and social cognition, including the Hayling test, tests of theory of mind (the faux-pas test and a false-belief test) and a task of recognition of emotions.

Results: Groups were not statistically different regarding age and educational level. Compared with controls, ALS performed worse in executive tests, with lower lexical fluency ($p < 0.05$), more errors in the Stroop ($p < 0.05$) and Wisconsin Card Sorting Test (number of errors ($p < 0.01$) and number of perseverations ($p < 0.02$)). ALS patients also had impaired performance in the false-belief test ($p < 0.02$) and Hayling test (total error score, $p < 0.03$), but there were no differences in the faux-pas test and recognition of emotions, in comparison with controls. ALS and bvFTD did not differ in the Hayling test and emotions recognition, but there were differences in tests of theory of mind, with ALS patients performing better than bvFTD patients (faux-pas and false-belief tests, $p < 0.05$ for both). bvFTD patients performed worse than controls in all cognitive measures.

Discussion and conclusions: ALS patients had an intermediate cognitive performance between bvFTD patients and healthy controls. Besides executive deficits, ALS patients also have impairment in functions related to social cognition, such as theory of mind. Despite the preliminary nature of the results, they indicate that cognitive dysfunction in ALS encompasses more than classical executive functions. Moreover, they reinforce the clinical and neuropsychological continuum across ALS and bvFTD.

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P96 COGNITIVE-BEHAVIORAL CHANGES IN AMYOTROPHIC LATERAL SCLEROSIS: NATURAL HISTORY AND IMPACT ON PATIENTS AND CAREGIVERS

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Keywords: Frontotemporal dementia(FTD), quality of life, caregiver burden

Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that exists on a clinicopathologic continuum with frontotemporal dementia (FTD). The natural history and impact of cognitive-behavioral deficits (CBDs) in ALS have not been rigorously characterized.

Methods: We performed longitudinal evaluations of CBDs in 95 ALS patients over 6 months using the ALS Cognitive-Behavioral Screen.™ Multiple regression was used to evaluate the association between CBDs and disease stage (ALS FRS-Revised), patient quality of life

(McGill QOL-SIS), and caregiver burden (Caregiver Burden Scale) after controlling for clinically important variables (age, education, gender, forced vital capacity, region of onset, depression, and pseudobulbar affect).

Results: At baseline (n=95), 62 (65%) participants were cognitively or behaviorally impaired and 6 (6%) met criteria for FTD. There was no association between the severity of cognitive-behavioral deficits and disease stage or patient quality of life. More pronounced cognitive deficits (beta=-1.4, p=0.04) and behavioral symptoms (-0.70, p<0.001) were associated with higher caregiver burden. Quality of life was reported to be lower in patients with more depressive symptoms (beta=-0.31, p<0.001) and more advanced disease (beta=0.10, p=0.014). Longitudinal data will additionally be presented at the conference.

Conclusions: CBDs are common in ALS and not predicted by the degree of motor weakness, which supports the use of routine neuropsychological screening. Cognitive-behavioral deterioration may have a greater impact on caregiver burden than functional impairment due to progression of motor weakness, but does not appear to significantly influence patient quality of life. Depression may be a treatable target to improve quality of life in patients with ALS. Better characterization of the natural history and impact of CBDs in ALS will be critical for designing future genotype/phenotype studies, screening recommendations, and targeted interventions to optimize quality of life for patients and caregivers.

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P97 ASSESSING THE EFFECT OF A GROUP THERAPY IN PARALLEL (SGP) FOR ALS PATIENTS AND THEIR CAREGIVERS

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Keywords: support group, anxiety, depression

Background: ALS is a rapidly evolving and short life expectancy neurodegenerative disease, with a strong emotional impact for those who suffer and those who live with it. The way a person copes with the continuously changing situation, will be the key to determine mood during illness. The efficacy of group therapy is known in many diseases, and for caregivers of ALS, but there is only some evidence about the group treatment for ALS patients. Facing this situation, Miquel Valls Foundation (FMQV) organizes Support Groups in Parallel (SGP), where different emotional aspects of the disease are simultaneously developed in adjoining rooms, one for patients and one for their caregivers. SGP took place during 6 consecutive weeks as closed groups, managed by 2 FMQV psychologists, one for each group.

Objectives: Assess the intra and inter SGP effectiveness, considering variables such as anxiety, depression and coping trauma strategies.

Methods: Descriptive and comparative cross-sectional study pre and post-test of 21 subjects, 14 caregivers (8 female and 6 male, average age 46, 46) and 6 ALS patients (3 female and 3 male, average age 59, 71).

The following parameters were evaluated: depressive symptoms with Beck Depression Inventory (BDI); anxiety using the State-Trait Anxiety Inventory (STAI); and posttraumatic growth through the Posttraumatic Growth Inventory (PTGI). The measurements were taken at the beginning and at the end of the SGP.

Results: We applied a descriptive statistical analysis and T-test (α 0.05). We observed a decrease in the average scores in the post-BDI and post-STAI, and an increase in post-PTGI in both groups (patient and caregivers). Taking into account the variances, we saw homogeneous scores in the group of caregivers, but not in the group of patients. We did not observe significant differences between men and women in both groups.

Discussion and conclusions: The scores show a slight improvement in the values of anxiety, depression and posttraumatic growth. To ensure the effectiveness of the SGP we would have to repeat the study with a bigger N and with a control group.

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P98 THE MISMATCH NEGATIVITY REVEALS SUBCLINICAL COGNITIVE DYSFUNCTION IN ALS PATIENTS

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Keywords: mismatch negativity(MMN), cognitive dysfunction, source analysis

Background: Central auditory processing is affected in a large number of different clinical conditions such as Amyotrophic Lateral Sclerosis (ALS). Cognitive deficits in ALS have been recorded using Event-Related Potentials (ERPs). The Mismatch Negativity (MMN) ERPs component is an index of auditory abnormalities, cognitive impairments and functional decline shared by different neurological disorders (1, 2). However, only a few studies have assessed MNN component in patients with ALS. ERPs source analysis could be a useful tool in investigating subclinical cognitive dysfunction, neural substrates and cerebral regions involved in MMN in ALS patients (3).

Objective: To describe MMN features in ALS patients; To determine and localize sources of electrical cognitive activities using a MMN auditory paradigm.

Methods: The MMN component was recorded in 17 patients with ALS (seven with spinal-onset, 11 with bulbar-onset). The auditory paradigm was validated in 24 healthy volunteers using 32 EEG channels montage. Only subjects with normal Brain Auditory Evoked Potentials (BAEPs) were included in the study. MMN Grand Average/Difference was computed and electrical sources were estimated by means of the Bayesian Model Averaging (BMA). Depression was assessed with Beck Depression Inventory. Clinical variables were evaluated with standardized scales for ALS group.

Results: MMN components were identified in 11 ALS patients and 16 healthy controls. A significant proportion of ALS bulbar-onset patients ($p \leq 0.05$) showed small or an absence of MMN component than the ALS spinal-onset patients. MMN was significantly different between patients and healthy controls for latencies and amplitudes. MMN major sources were localized in fronto-temporal and fronto-central cortical areas in healthy subjects. For patients, MMN main sources were limited in frontal areas with right lateralization.

Conclusions: The lack of MMN in about 1/3 of patients with ALS could indicate poor auditory discrimination performance. Our findings suggest that the MMN indexes a deterioration in the auditory sensory memory suggest a shorter duration of the memory trace in ALS patients similar in the process of dementia. The cognitive impairments found in the neuroimaging studies evince the fronto-temporal lobe dysfunction reflecting cortical degeneration beyond the motor areas that could be associated to an early stage of fronto-temporal dementia. The MMN is a useful non-invasive tool for investigating of auditory processing deficits in ALS disorder.

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P99 COGNITIVE SPECTRUM OF CHINESE PATIENTS WITH SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: frontotemporal lobe degeneration, cognition impairment, neuropsychology

Background: The cognitive spectrum of Chinese patients with sporadic amyotrophic lateral sclerosis (sALS) still waits to be documented.

Methods: Patients with sALS were consecutively enrolled and comprehensive neuropsychological tests covering memory, executive function, attention, language, visuospatial function were administered. Meanwhile, the same neuropsychological tests were performed to 76 age- and education- matched healthy controls (HC).

Results: 106 Patients with sALS (51.310.4 years old) were categorized into 5 subtypes: 86 (81.1%) with normal cognition (ALS-NC); 12 (11.3%) with executive cognitive impairment (ALS-ECI); 3 (2.8%) with non-executive cognitive impairment (ALS-NECI); and 5 (4.7%) with frontotemporal lobe degeneration (ALS-FTLD). Under the same criteria, 2 (2.6%) and 1 (1.3%) HC were diagnosed as ECI and NECI, respectively. The proportion of ECI was significantly higher in non-demented ALS than that in HC (p=0.024), but it was not for NECI (p=0.463).

Compared to HC, non-demented ALS performed significantly worse in verbal fluency (p=0.001); backward digital span (p=0.033); episodic memory (p=0.003); the Clock Drawing Test (p=0.027); and the Symbol Digit Modalities Test (p=0.003). Age, gender, education level, onset type, disease duration, general function status and progression rate were not significantly different among ALS-NC, ALS-ECI and ALS-NECI, except for bulbar function where it was significantly lower in ALS-FTLD than that in ALS-NC (p=0.001).

Discussion: The diagnosis of FTLD is not fully based on cognitive batteries. As a result, the rates of FTLD fell below 5% in both our cohort and the previously reported Korean study, whereas the prevalence of FTLD reached above 10% and up to 15% in Europe and the US. Rather lower prevalence of C9orf72 carriers might serve as another important reason for this difference. Age distribution could be another possible explanation: patients under 45 years old, accounting for 26% of our cohort, seem to be free from cognitive impairment. The mean age of Caucasians studies ranged from 58.8 years 14.4 to 67.0years 9.9, an age range much older than both ours,= and the Korean study (51.3 years 10.4 and 55.7 years 10.7), respectively. Based on these facts, it is likely that aging and neurodegeneration synergistically erodes the cognitive reserve of ALS patients, or the function of the

frontal and temporal lobes is inherently spared in early onset ALS.

Conclusion: Comorbid FTLD occurred in around 5% of Chinese sALS cases. Differences of genetic background and unique age distribution of Chinese sALS patients might be the reasons for the relatively low rate of comorbid FTLD. Despite these, compared to HC, executive function remains a vulnerable area of Chinese sALS.

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Theme 6 Respiratory and Nutritional Management

P100 COMPARISON OF SEVERAL RESPIRATORY ASSESSMENTS IN ALS

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Keywords: hypoxia, autonomous dysregulation, niv

Background: In ALS all three components of the respiratory system (inspiratory, expiratory and bulbar muscles) decline, leading to progressive decline in vital capacity (VC) and increase in breathing. Neuromuscular disorders may also be associated with obstructive or central sleep-disordered breathing.

Objectives: Comparison of different assessments of respiratory function in ALS.

Methods: Polygraphy records, pulmonary function tests and clinical data were reviewed for 131 ALS patients. The respiratory polygraphy (Somnoscreen from SOMNOmedics Randersacker, Germany) recorded: pulse oximetry; oronasal pressure; and flow using thermistor; chest/abdomen movements by impedance plethysmography; body position by position sensor; snoring by microphone; heart rate; and oxygen saturation (SpO_2). The pulmonary function tests included: vital capacity (VC, %predicted); forced vital capacity (FVC, % predicted); and the maximal static inspiratory mouth pressure (Pimax).

Results: We determined how the clinical symptom dyspnea is reflected in different values of pulmonary function tests and polygraphy. The patients group complaining of dyspnea vs. not complaining of dyspnea had a significantly lower ALSFRS-R ($P<0.001$); lower VC ($P = 0.002$); lower FVC ($P = 0.008$); lower SpO_2 ($P = 0.001$); lower PaO_2 (0.006); and higher AHI ($P < 0.00$) while other values such as Pimax; baseline SpO_2; the nadir of SpO_2; mean SpO_2; and $PaCO_2$ did not significantly differ. Nocturnal hypoventilation was observed in 67% (32 bulbar, 56 limb onset, total n=88) independently of the ALSFRS-R. Patients with nocturnal hypoventilation were characterized by a significant lower VC (P=0.018), lower FVC (P=0.019), and lower Pimax (P=0.008). However, also in the absence of nocturnal hypoventilation eight patients had a VC below 50% predicted. On the other hand in 60 patients with nocturnal hypoventilation the VC was over 50% predicted.

Discussion: This is the first study providing comprehensive data on pulmonary function test and nocturnal polygraphy in a large cohort of patients with ALS. The ALSFRS-R and its respiratory question correlated with the decline of VC, FCV and changes in nocturnal BGA. However, the absence of dyspnea doesn't rule out a relevant respiratory impairment, since 30% of the ALS patients not complaining of dyspnea had a FVC lower than 75% predicted. This is of special importance since a FVC of 75% predicted rather than 50% is discussed as threshold for the initiation of NIV. The absence of nocturnal hypoventilation does not necessarily indicate normal respiratory function, since FVC ranges in this group from 25% till 123% of predicted. Moreover we found that sleep related respiratory events were more common in the early stages of disease with a progressive decline in the number of events (AHI, hypopnea, apnea) during disease course.

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P101 THE DEVELOPMENT OF A MULTIDISCIPLINARY CLINIC TO IMPROVE RESPIRATORY CARE OF PEOPLE WITH MND/ALS IN MEDWAY AREA

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Keywords: non-invasive ventilation, multidisciplinary clinic, quality of life

Background: There is increasing awareness of the role of respiratory support for people with motor neurone disease (MND)/amyotrophic lateral sclerosis (ALS) to improve both quality of life and survival. In the UK the National Institute of Health and Care Excellence Guidance on the use of Non-invasive ventilation (NIV) in MND in 2010 suggested a multidisciplinary team approach and close collaboration between services. In Medway all patients attending the MND clinics are monitored regularly for the symptoms and signs of respiratory dysfunction and joint clinics have been developed including both palliative care and respiratory medicine, to discuss and monitor the use of NIV.

Objectives: The aim of the study is to evaluate a new multidisciplinary approach for NIV.

Methods: The details and the outcomes of all patients seen within the joint palliative medicine/respiratory medicine clinic have been evaluated over a three year period.

Results: 13 patients have been considered over the last 3 years: 9 male, 4 female; mean age 57 years (range 44 – 74 years); and mean time from first symptom to consideration of NIV was 20.5 months (range 1 – 61 months). 12 patients have started on NIV successfully at home; 15% of all the patients are cared for with MND/ALS in the area with repeated visits and support from the Specialist Respiratory Nurse, facilitating the use of NIV for patients who were initially very anxious. By April 2015 six had died and the mean time of use of NIV was 12.5 months (Range 1-53 months). Two were withdrawn from NIV at their request and the others died without needing withdrawal. Six patients were still alive and have been using NIV for a mean use 17.7 months (Range 1-43 months).

Discussion: This joint approach has allowed people with MND/ALS to start NIV, with improvement in quality of life. The discussion has allowed a wider consideration of the benefits of NIV and the discussion of disease progression and the possible consideration of later withdrawal, as recommended by the NICE Guidance. The joint clinic has allowed a clearer approach to patient care with home commencement of NIV with a more comprehensive service to be provided with increased support of patients and their families, and increased compliance with the intervention, leading to improved quality of life.

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P102 WORSENING OF RESPIRATORY FUNCTION IS PREDICTIVE OF PROGNOSIS OF ALS EVEN AT A VERY EARLY STAGE

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Keywords: respiratory insufficiency, ALS prognosis, SVC

Introduction: Most ALS patients will die of respiratory failure, with a median interval of 3 years after onset. International criteria for starting non-invasive ventilation (NIV) are well defined. Conversely, the role/impact of ALS clinical and functional parameters both on the decision of NIV and prognosis is not well defined.

Methods: 135 ALS patients were studied and followed on a regular basis. Clinical data were collected, including: age of ALS onset; site of onset; ALS duration; and delay for NIV. Functional data collected were: ALSFRS; SVC; FVC; and weight. From the initial visit for the diagnosis, these parameters were followed at months 6, 12 and 18.

Results: In the study there were 88 men and 47 women. Mean age of onset of ALS was 60.4 years and mean ALS duration was 35 months. In this population, the delay for starting NIV strongly correlated with ALS duration ($R2=0.69$, $p<0.0001$). We studied the influence of SVC changes (% SVC decline at 6, 12 and 18 months) on ALS prognosis and found a significant correlation between SVC changes at 6, 12 and 18 months and ALS duration, with the strongest correlation at 18 months ($R2 = 0.30$, $p = 0.018$). Correlations were also significant but weaker for FVC. As expected, ALSFRS changes (% ALSFRS decline at 6 months) correlated with ALS duration, the patients with the most rapid decline of ALSFRS had the worst prognosis ($R2=0.19$, $p<0.001$). However, there was a stronger correlation between SVC changes and ALSFRS decline than between ALSFRS decline and ALS prognosis ($R2=0.24$, $p<0.0001$). Surprisingly, weight loss did not correlate either with ALS prognosis, delay for NIV or SVC changes.

Conclusions: As soon as 6 months after the first visit for ALS diagnosis, SVC changes are predictive of prognosis, as are ALSFRS changes, but not weight loss. As already suspected clinically, the delay for starting NIV is also predictive of a bad prognosis of ALS and this is the stronger correlation found in this study. Respiratory function has to be monitored during the day and during the night at every stage of the disease as early changes may help physicians to better inform the patients about the potential progression of their disease. We suggest that studies should focus on demonstrating that NIV should be started earlier in the disease process, earlier than what is done with the actual criteria, to try to optimize prognosis.

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P103 MULTIDISCIPLINARY RESPIRATORY CARE SUPPORT TEAM IMPROVES RESPIRATORY MANAGEMENT OF INPATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: multidisciplinary respiratory care support team(RST), TPPV, NPPV

Objectives: We performed a retrospective hospital-based study to clarify the clinical effectiveness of a multidisciplinary respiratory care support team (RST) for hospitalized patients with Amyotrophic Lateral Sclerosis (ALS).

Background: We provide long-term medical care for neuromuscular disease patients at the neuromuscular-disease center hospital in the Hokuriku area of Japan. To improve each patient's prognosis or to reduce respiratory complications, we started a multidisciplinary respiratory care support team (RST) since 2007.

Methods: We retrospectively reviewed medical records of inpatients with ALS to elucidate the RST activity from 2010 to 2014. The prognosis after RST intervention of 28 patients was investigated. Twenty patients out of those

28 were assisted by a mechanical ventilator before RST consultation.

Results: We were able to pick up 106 conference records from 2010 to 2014. RST intervention was performed for 28 cases. Thirteen of 28 patients had ended RST intervention. The main reasons for having ended intervention was discharge from hospital having achieved the initial aim of RST intervention. Others were death in pneumonia or respiratory failure. Pneumonia frequency reduction or improving airway clearance was observed in 9 patients who were continuing the intervention by RST or by floor nursing team.

Conclusion: From this retrospective study, the improvement of respiratory conditions like pneumonia frequency reduction etc, can be expected even for severely disabled ALS patients by systematic RST intervention. We concluded multidisciplinary RST intervention can be clinically effective.

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P104 AIR STACKING EFFECT ON MOTOR NEURON DISEASE / AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: breathing exercises, respiratory muscles, respiratory care

Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease, characterized by progressive muscular paralysis and caused by degeneration of motor neurons in motor cortex, brainstem and spinal cord. Denervation of the corresponding motor neurons causes muscle weakness, which is insidious, at first asymmetric and afflicts distally or proximally. As the disease progresses, combination of signs and symptoms of upper and lower motor neurons (spasticity, atrophy, fasciculation) and bulbar damage (dysphagia, dysarthria, dyspnea, sialorrhoea) are common. Respiratory failure and respiratory complications are the main cause of death in ALS. Inspiratory muscle weakness leads to loss of forced vital capacity (FVC), which is most likely variable and linear, ranging from 2.5% to 8.3% per month. It also decreases peak cough flow (PCF) in ALS patients when associated with weakness of bulbar muscles and expiratory muscles; thus, increasing the risk of respiratory complications, eg bronchoaspiration, pneumonia and atelectases. It is necessary, thus, to provide regular insufflation by means of stacking volumes of air by closing the glottis to achieve maximum inflation capacity (MIC). It is believed that such technique optimises lung function and lung compliance of patients by means of increasing PCF and MIC.

Objective: In this study, we aimed to evaluate the air stacking effect on PCF, MIC, PFT measures in MND/ALS patients.

Method: A cross-sectional study was performed in the department of research on neuromuscular diseases, Federal University of São Paulo (UNIFES), Brazil. Based on the El Escorial criteria, subjects with a confirmed diagnosis of ALS and able to conduct pulmonary function tests were included, patients were already trained and oriented concerning air stacking. In this study, 17 participants took part: 10 (58%) men; 7 (42%) women, with average age of 50.41±7.70. We applied FVC, PCF tests and MIC, PCF tests soon after performing the air stacking. We tested the data normality by using the KS test; afterwards, we applied the paired t-test, with a significance level of $p<0.05$.

Results: When compared to FVC, MIC increased (0.469 ml / $p<0.05$); PCF also increased (31 l/min / $p<0.05$) after applying air stacking technique.

Conclusion: Air stacking technique increases both MIC and cough in ALS patients.

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P105 AVERAGE VOLUME ASSURED PRESSURE SUPPORT (AVAPS) IN ALS PATIENTS WITH PURE RESPIRATORY INVOLVEMENT: A RANDOMIZED DOUBLE-BLIND CROSSOVER TRIAL

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Keywords: noninvasive ventilation (NIV), AVAPS, adherence

Background: Non-invasive ventilation (NIV) can improve survival and quality of life in people with ALS. However, there is no data comparing different modes of NIV (volume-targeted versus pressure-limited). Factors associated with poor adherence include bulbar dysfunction and cognitive impairment. In this study, we sought to determine if the mode of NIV played any role in successful use of NIV. To avoid confounding variables, we limited our study to non-demented patients with clear respiratory symptoms, and no bulbar involvement.

Methods: This was a crossover design study of 14 patients. We compared modes of NIV using a single unit with dual mode capabilities. Enrolment criteria included FVC < 65% of predicted and patient-reported dyspnea and orthopnea. FRS bulbar score was limited to 11 or 12. Patients were provided a 2-week lead-in period for

acclimation in one mode, and then were crossed to the alternative NIV mode for 6 weeks, and subsequently back to the initial NIV mode for the remaining additional 6 weeks. Adherence was defined as > 4 hours of use per night. Our primary end points were adherence to pressure-limited NIV or volume-targeted NIV. We also measured sleep quality, dyspnea, orthopnea, and quality of life on both modes.

Results: Twelve of 14 patients completed the full study. There was no statistically significant difference between the two modes of therapy for any measure. For our primary measure of adherence, all patients were able to use Vol-NIV for a total of 85.41 hours, an average of 7.12 hours and Press-NIV for 86.33 hours, an average of 7.19 hours. (Mean -0.08, sd 1.56, t-stat -0.17, 2-sided p=0.43). However, the second intervention period was associated with increased hours of use, independent of treatment mode.

Conclusions: 1) In this population of patients with "pure" respiratory failure, all patients adhered to NIV therapy, independent of treatment modality. This is far higher than reported rates in a general ALS population. This implies that factors such as patient selection, practitioner experience, and close follow up are more important than treatment modality; 2)The increased adherence in the second treatment period could suggest increased need as the disease progresses or that learning plays a role in adherence.

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P106 A DECADE OF DIAPHRAGM PACING FOR ALS/MND: OVERALL SURVIVAL AND CURRENT MANAGEMENT OF DIAPHRAGM PACING

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Keywords: diaphragm pacing, hypoventilation, respiratory

Background: Diaphragm Pacing (DP) was designed to overcome the loss of upper motor neuron (UMN) control of the diaphragm when there are intact diaphragm motor units to stimulate. This report outlines a decade of utilization of DP in ALS/MND.

Objective: Report absolute survival of all patients implanted with DP. Outline current pre-operative analysis and post-operative management of DP.

Methods: Analysis of all consecutive patients with ALS/MND at a single site, evaluated or implanted with DP, under 4 separate IRB and/or FDA prospective trials or databases. All patients underwent some or all of the following pre-operative diaphragm neurophysiologic testing: phrenic nerve conduction studies; diaphragm fluoroscopic sniff studies; diaphragm ultrasound thickness analysis; and inspiration/expiration chest x-rays. All patients underwent the critical final intra-operative direct diaphragm stimulation during diaphragm mapping.

Results: From 3/2005 to 3/2015, 214 patients were implanted. Overall median survival was 21.8 months (95% CI 18.1, 26.0 months) post implantation. Peri-operative 30 days morbidity of tracheostomy or death involved 3 patients (1.4%) in which excessive uncontrolled secretions were involved. Operative assessment of diaphragm movement under direct stimulation did not always correlate to pre-operative testing. Over the last 18 months, 20% of patients assessed pre-operatively for implantation were not taken to surgery because diaphragm neurophysiologic testing did not reliably document stimulatable diaphragm motor units to undertake the slight risk of surgery. Nevertheless, 10% of patients still going to surgery were not implanted. The now routine post-operative analysis of the electromyography of the implanted diaphragm electrodes has identified instability of respiratory control when patients have intact diaphragm motor units that DP can therefore manage with stimulation (1). Active management of DP to maximize ventilation and prevent hypercarbia include: progressive utilization to 24 hours a day including sleep; increasing DP setting and respiratory rate; and utilizing DP in conjunction with non-invasive ventilation (NIV). Patients with intact diaphragm motor units and associated phrenic motor neurons with no UMN control are instructed that rapid removal of DP can cause rapid respiratory decline. This is important for end of life management.

Conclusion: DP can be safely implanted with a low pre-operative morbidity. Median survival of 21.8 months for all patients implanted is similar to the pilot results (2). Identifying appropriate ALS/MND patients with stimulatable diaphragm motor units involves pre-operative screening but ultimately careful discrimination via laparoscopic evaluation. DP is never warranted when there is no response to direct stimulation at surgery. Excessive secretions with aspiration are a relative contra-indication. Appropriate pre-operative testing, intra-operative assessment and active DP management will provide the optimum care for ALS/MND patients.

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P107 DIAPHRAGM PACING MIGHT IMPROVE SLEEP IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: NIV, diaphragm pacing, sleep

Background: Respiratory insufficiency is a critical problem in amyotrophic lateral sclerosis (ALS) patients. Noninvasive ventilation (NIV) prolongs survival in ALS but its effect on quality of life is unknown. Diaphragm pacing (DP) is used to augment respiration when patients have intact diaphragm motor units and has been applied in several neurological disorders. It can be used in conjunction with NIV, but this is the first report of DP as primary treatment in ALS for respiratory insufficiency and its effect on sleep from Japan.

Objectives: The aim of this study was to investigate the efficacy of DP for sleep condition in ALS patients. We started DP with NeuRx Diaphragm Pacing System^{R} (NeuRx DPS, Synapse Biomedical, Ohio, USA) for 5 Japanese ALS patients without positive-pressure mechanical ventilation (3 men and 2 women, aged 59.6±9.6 years). We implanted DP electrodes into the diaphragm close to the phrenic motor point laparoscopically under general anesthesia according to a previous report (1) and initiated DP for 24 hours a day. We evaluated respiratory function and sleep condition with full-night laboratory polysomnographic recordings (PSG) before and 6 months after the administration of DP. PSG was performed with the stimulator turned off.

Results: Evaluations were completed in 4 patients. One patient withdrew due to replacement of mechanical ventilation with tracheostomy. According to the disease progression, vital capacity (% predicted, sitting) decreased and PaCO2 (mmHg) increased (VC: 61.1 ± 7.0 to 37.9 ± 6.0, PaCO2: 37.9 ± 0.7 to 41.6 ± 3.3). Regarding sleep condition, % time of total sleep time in Stage N1-N2 and REM sleep remained similar during the study period (Stage N1-N2: 77.7 ± 4.6 to 74.2 ± 8.3, REM sleep: 21.6 ± 5.1 to 25.0 ± 7.9). However, wake after sleep onset (WASO, min) was reduced 220.6 ± 108.0 to 144.5 ± 44.0. In addition, both arousal index (AI, per hour) and apnea-hypopnea index (AHI, per hour) improved (AI: 29.4 ± 17.7 to 16.0 ± 11.6, AHI: 16.8 ± 12.4 to 6.8 ± 4.6).

Discussion and conclusion: As previously reported (2), NeuRx DPS might improve sleep in ALS patients in the early periods from the administration of DP. Optimum therapy may involve both DP and NIV as the patient's disease progresses.

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P108 ANALYSIS OF FUNCTION AND SURVIVAL IN ALS PATIENTS WITH DIAPHRAGM PACING USING VIRTUAL CONTROLS

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Keywords: predictive model, diaphragm pacing, PRO-ACT database

Background: The *NeuRx DPS*® diaphragm pacing system (DPS), implanted through a minimally invasive laparoscopy, has been approved by the US FDA for treating ALS patients who are experiencing hypoventilation and have diaphragms that can be electrically stimulated. Additional studies are ongoing to improve the class of evidence and help select patients that would be expected to benefit. With an approved device, enrollment in a randomized trial and interpretation of results on small samples is difficult. To that end, we developed predictive model-based comparators using the large PRO-ACT dataset to analyze survival and function in DPS implanted ALS patients and sub-cohorts.

Objectives: To develop predictive functional disease progression and survival models for ALS and apply these models to patients who have previously been implanted with *NeuRx DPS*® during the clinical trial NCT00420719.

Methods: We used the PRO-ACT ALS database to develop a random forest (RF) model to predict disease progression as measured by ALSFRS-R and a gradient boosting model (GBM) survival model to predict survival at time points up to fifteen months into the future.

The RF model to predict function was trained on 1100 records in PRO-ACT containing complete records for ALSFRS-R and FVC. Variables used included: baseline ALSFRS and slope; subscores; time since disease onset and diagnosis; baseline FVC and slope; weight; age; bulbar/limb onset; study arm; gender; and Riluzole use. Model performance was assessed via K-fold internal cross validation, external validation, and comparison of RMSD among other candidate models.

The GBM survival model was built using 4633 PRO-ACT records, including 1427 deaths, that contained both FVC

and either ALSFRS or ALSFRS-R. Concordance index and Brier scores were used to compare candidate survival models including Cox proportional hazards and random survival forest models against internal and external validation datasets.

Finally, functional and survival predictions were made from the time of DPS implantation for the 106 patients in the DPS trial and used to generate virtual control groups against which the actual observations were compared.

Results: The ensemble ALSFRS-R disease progression model chosen did not detect a significant difference in total ALSFRS-R score between predictions and observations in DPS patients. In contrast, we observed a significant improvement when we compared fifteen months predicted survival of DPS implanted patients, 40% survival probability, to their observed Kaplan-Meier curves, 80% survival probability ($p<1x10^{-5}$).

Discussion and conclusions: Our findings support the hypothesis that DPS improves survival while having a minimal effect on patient functional capabilities as measured by total ALSFRS-R. Additional studies of sub-cohorts will provide further evidence of DPS responders to aid in clinical selection of patients. These studies may be aided by using the GBM predictive model as a virtual control arm.

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P109 FACTORS ASSOCIATED WITH PATIENT COMPLIANCE WITH NON-INVASIVE VENTILATION IN PATIENTS WITH ALS

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Keywords: non-invasive ventilation, compliance, progression

Background: Hypercapnic respiratory failure, hypoxemia, pneumonia and aspiration are the major factors contributing to mortality in ALS. Non-invasive ventilation (NIV) in ALS has been shown to improve survival and also quality of life, while compliance with NIV treatment is an important predictor of survival. For patients requiring NIV, Medicare requires a data report from the patient's device which documents use of the NIV device for at least 4 hours per night during the first 90 days after starting treatment. However, the rationale for the use of a 4-hour minimum as indicating compliance in ALS has been questioned by some.

Methods: We studied a cohort of 165 patients with ALS who were prescribed non-invasive ventilation at Northwestern Memorial Hospital. Data were collected prospectively over three visits, and patients were divided into two groups for analysis: 1) those that were previously established on NIV at the start of the study period and 2) those who had initiated NIV during the study period.

Results: 165 patients were included in the study. Mean age at first visit was 59.2 years, 68 (41.2%) were female. For patients established on NIV at their first study visit, the mean number of hours of NIV use per day was 7.3 hours, compared with 5.2 hours per day for patients who recently commenced NIV ($p<0.0001$).

For the second visit, established NIV patients had a mean of 9.4 hours use compared with 6.5 hours for those who recently initiated NIV ($p=0.004$). For the third visit, mean usage was 10.45 hours for established patients and 7.3 hours for recently-initiated patients ($p<0.001$). Significant increases in hours of NIV usage were seen in both established NIV patients and new NIV patients from the first to the third visit ($p=0.03$).

Conclusion: Patients with ALS who commence NIV treatment for respiratory failure may initially demonstrate poor compliance as defined by Medicare recommendations. However, these recommendations may not be suitable for patients with ALS, as over time this compliance is seen to improve significantly. Further study is needed to establish appropriate NIV compliance recommendations for patients with ALS.

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P110 PROLONGED SURVIVAL OF NON-INVASIVE AND INVASIVE VENTILATION IN JAPANESE PATIENTS WITH ALS

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Keywords: NIPPV, tracheostomy, prognosis

Objective: Non-invasive ventilation (NIV) and tracheostomy/invasive ventilation (TIV) in patients with ALS are common therapeutic options in Japan. NIV has been considered a first–line treatment for respiratory distress since 2000. In this study we evaluated the prognostic factors for survival with NIV and TIV.

Methods: Survival data until tracheostomy or death was obtained from 160 Japanese patients.

Results: A total of 74 patients received ventilation supports: 22 patients underwent NIV only; 32 patients underwent TIV only; 20 patients underwent TIV after NIV. Both TIV and NIV prolonged median survivals as compared to no intervention (74 months vs 48 months vs 32 months; $p<0.001$). The study showed a low, 30 day mortality rate after NIV initiation, indicating general good adaptation to NIV in our series. Cox proportional modelling confirmed that age under 65 years was advantageous for longer survival after TIV. Median survival time until death after TIV was longer (89 month) for patients at home than those in a hospital (61 month, $p=0.007$). Post-NIV survival showed no significant differences in age, gender, or the presence of spouse, but patients with bulbar palsy at the time of initiation of NIV showed significantly

shorter survival than those without it, unrelated to nutritional state. At any time of pulmonary function state, NIV prolonged longer survival in patients with ALS. Significant prolonged survival from symptom onset was found in patients with over 30% of FVC, as compared to those under 30%. Progression rate at diagnosis calculated by ALSFRS-R was related to post-NIV period.

Conclusions: NIV offered advantages of being non-invasive, having no risk and being easy to introduce or discontinue. The frequency of use of NIV significantly increased after 2000. Various factors impact the survival of patients after TIV (including age, home) and NIV (bulbar symptom, progression rate). The present study provides factors related to the decision-making process and survival in post-NIV and TIV.

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P111 JAPANESE NEUROLOGISTS RECOMMENDATIONS TO THEIR PATIENTS AND PERSONAL CHOICES TO TRACHEOSTOMY WITH INVASIVE MECHANICAL VENTILATION (TIV) DIVERGE BUT NOT TO NON-INVASIVE MECHANICAL VENTILATION (NIV)

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Keywords: mechanical ventilation, patient's choice, doctor's choice

Background: It is reported that American neurologists seldom recommended TIV and most would not choose TIV themselves. On the other hand Japanese neurologists were more likely to recommend TIV to their patients than choose it for themselves (1). What is the reason for this difference? There are several hypotheses but the reason is not known yet.

Objectives: To clarify the reason for divergence between Japanese neurologists' recommendations and personal choices.

Methods: We sent out an anonymous questionnaire with 45 questions to 5144 board certified neurologists. We asked about their clinical experiences and thoughts in connection with palliative care and patient's right. 1490 neurologists returned the questionnaire (27% response rate). We analyzed the following four questions: Question 5-2 'In your own practice, about what % of patients receives TIV?'(1.Almost 100%; 2.∼80%; 3. ∼50%; 4.∼20%; 5.∼20%; .6.∼10%; 7.Others). Question 7-2 'If you have ALS would you choose TIV for yourself? 1. Yes; 2. Probably yes; 3.probably no; 4. No; 5. cannot decide). Question 5-1 and 7-1 are same questions regarding NIV respectively.

Results: Question 5-1: (1)0%; (2)4%; (3)14%; (4)25%; (5)15%; (6)32%; (7)10%. Indicating 43% of neurologists answered that more than 20% of patients choose TIV (1+2+3+4). Question 5-2: (1)3%; (2)9%; (3)28%; (4)48%; (5)12%. Indicating that only12% answered that they would choose TIV. Question 5-1: (1)5%; (2)12%; (3)23%; (4)19%; (5)8%; (6)23%; (7)10%. Indicating 59% of neurologists answered that more than 20% of patients choose NIV. Question 7-1: (1)12%; (2)34%; (3)20%; (4)23%; (5)11%. Indicating that 46% of neurologists answered that they would choose NIV.

Discussion: As previously reported, regarding TIV, Japanese neurologists' recommendations and personal choices diverged. In contrast, their recommendations and personal choices for NIV did not diverge. In Japan costs and accessibility are the same for both TIV and NIV. Therefore, what is the reason for this difference? The differences between TV and NIV are: 1) TIV requires invasive procedure (Tracheostomy); 2) NIV can be withdrawn by his/her own decision; 3) Only with TIV is the voice lost. The risk of tracheostomy is limited and a voice will be lost in the time course of disease, therefore it seems likely that the most likely reason for the differences is 2). In Japan, generally speaking public opinion is 'To live is splendid' and there are many ventilated patients as a role model covered by the mass media. The assumption from this study is that those factors make more patients choose TIV in Japan. However, neurologists may have more concrete ideas of living on TIV than patients do which could make them reluctant to choose TIV while withdrawal is not legally supported.

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P112 RELATIONSHIP BETWEEN ADVERSE CLINICAL SIGNS AND PROGRESSION OF COMMUNICATION IMPAIRMENT IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS ON TRACHEOSTOMY INVASIVE VENTILATION

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Keywords: tracheostomy invasive ventilation(TIV), communication impairment, adverse clinical signs

Background: Patients with amyotrophic lateral sclerosis (ALS) on tracheostomy invasive ventilation (TIV) often develop serious communication impairment and show adverse clinical signs with disease progression.

Objective: To clarify the relationship between adverse clinical signs and stage progression of communication impairment in ALS patients on TIV.

Methods: We enrolled sixty ALS patients using home TIV. We prospectively followed up each patient from 2005 to 2014. At the last time of investigation, the patients were classified according to previously reported staging of communication ability (stage I–V). Data were collected on sex, age at onset, total disease durations, durations from onset to TIV, and durations on TIV. We also assessed time lengths from onset to the time when patients developed oculomotor impairment or became totally quadriplegic. We assessed sixteen clinical adverse signs as follows: fatigability of eye movement; dry eye; drooling or dry mouth; macroglossia; unstable blood pressure; disturbance of thermoregulation; dysuria; nausea; unstable blood glucose; otitis media; pneumonia, urinary tract infection; cholelithiasis; tracheal granuloma; urinary stone; and decubitus. The Cox proportional hazard model was used to elucidate which adverse signs were correlated with progression of communication impairment.

Results: Forty-one patients remained at stage I–III, and 19 patients developed into stage IV–V at the last of investigation. Multivariate Cox analyses showed that the time lengths from onset to the appearance of oculomotor impairment, unstable blood pressure, disturbance of thermoregulation and dysuria had significant effects on progression of communication impairment to stage IV–V.

Conclusion: The oculomotor and autonomic disturbances are considered not to be mere complications due to long TIV use but to be related with progression of ALS. These results are consistent with previous neuropathological reports in advanced ALS patients in stage V, which showed multisystem neurodegeneration involving the oculomotor nuclei and limbic system.

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P113 INTRODUCTION OF NONINVASIVE VENTILATION AND MECHANICALLY ASSISTED COUGHING IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: forced vital capacity, peak cough expiratory flow, mechanically assisted coughing

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

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

Methods: A total of 40 patients met the revised El Escorial criteria. We selected patients with a %FVC of <50% or a PECF of <270 L/min. The mean age (SD) of the patients was 59.4 $\pm$ 15.0 years in the BO (n = 10) group and 66.1 $\pm$ 8.8 years in the SO (n = 13) group. We evaluated the PCEF approximately every 3 months. The PCEF was determined three times per measurement, and the maximum value was taken as the PCEF. In addition, the body mass index (BMI) and the Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) score were also studied.

Results: A total of 23 patients were enrolled in this study. A decline in the PECF (<270 L/min) prior to a decline in %FVC (<50%) was observed in 7 and 10 patients in the BO and SO groups, respectively. However, with regard to the speech item on the ALSFRS-R, BO patients showed more speech impairment than SO patients (2.1 $\pm$ 0.9 vs. 3.3 $\pm$ 0.7; P = 0.04). No significant difference was observed in the BMI between the 2 groups.

Discussion: According to the Report of Quality Standards Subcommittee of the American Academy of Neurology, one of the parameters for considering NIV is a %FVC of <50% and the parameter for MAC is a PCEF of <270 L/min. NIV and MAC are important for improving respiratory function and clearing upper airway secretions. Patients in the BO group as well as most patients in the SO group showed a decline in the PCEF (<270 L/min) prior to a decline in the %FVC (<50%), although bulbar symptoms on the ALSFRS-R was different between the 2 groups.

Conclusion: The results of this study suggest that MAC may be introduced earlier than NIV in patients with both BO and SO.

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P114 LARYNGEAL RESPONSE PATTERNS TO MECHANICAL ASSISTED COUGH IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: mechanical insufflation-exsufflation (MI-E), larynx, laryngoscopy

Background: Most patients with amyotrophic lateral sclerosis (ALS) are treated with a mechanical cough assist device using the mechanical insufflation-exsufflation (MI-E) technique to improve cough and prevent lung infections. The technique often fails in ALS with bulbar involvement, allegedly due to upper airway malfunction. Laryngeal collapse during exsufflation has been proposed to explain ineffectiveness of MI-E in bulbar ALS (1).

Objectives: We have studied laryngeal response patterns during MI-E in detail with laryngoscopy, aiming to unravel information that could lead to better treatment.

Methods: We conducted a cross-sectional study of 20 patients with ALS and 20 healthy age and gender matched volunteers. We used video recorded flexible transnasal fiberoptic laryngoscopy during ongoing MI-E that was performed according to a standardized protocol applying pressures of ±20 to ±50 cmH2O. Laryngeal movements were assessed from video files. The type of ALS and the presence of upper and lower motor neuron symptom characteristics were determined.

Results: At the supraglottic level, all patients with ALS and bulbar symptoms (n=14) adducted their laryngeal structures during insufflation. At the glottic level, an initial abduction followed by a subsequent adduction was observed in all ALS patients both during insufflation and exsufflation. Hypopharyngeal constriction during exsufflation was observed in all study subjects, most prominent in patients with ALS and bulbar symptoms. Healthy subjects and patients with ALS and no bulbar symptoms (n=6) managed to coordinate their cough well during MI-E.

Discussion: Hypopharyngeal constriction during exsufflation was observed in all study subjects. This phenomenon cannot by itself explain treatment failure in bulbar ALS as proposed (1). The adduction response observed during insufflation precludes air-filling of the lungs, leading to discomfort and subsequent inefficient exsufflation and cough with no effect on airway clearance. Laryngeal adductor reflex circuit may be hyperresponsive or dysregulated in ALS patients and therefore lead to inappropriate laryngeal closure, comparable to observations made in patients with Parkinson's disease or brain stem compression (2, 3).

Conclusion: Laryngoscopy during ongoing MI-E in patients with ALS and bulbar symptoms revealed laryngeal adduction especially during insufflation as well exsufflation, severely compromising the size of the laryngeal inlet in some patients. We propose that individually customized pressure and flow settings can extend the use of non-invasive MI-E in ALS.

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P115 VOLUNTARY COUGH AIRFLOW PREDICTS PENETRATION/ASPIRATION STATUS IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: Screen, Dysphagia, Cough

Objective: Dysphagia and aspiration are prevalent in amyotrophic lateral sclerosis (ALS) and contribute to malnutrition, aspiration pneumonia and death. Early detection is critical to ensure maintenance of safe oral

intake and optimal pulmonary function. The purpose of the current study was to determine the discriminate ability of voluntary cough airflow measures in predicting the presence of penetration/aspiration in ALS patients.

Methods: 70 individuals with ALS (El-Escorial criteria) completed voluntary cough spirometry testing and underwent a standardized videofluoroscopic swallowing evaluation (VFSE). A rater, blinded to aspiration status derived six objective measures of voluntary cough airflow and airway safety evaluated using the validated Penetration Aspiration Scale (PAS). A between groups ANOVA (safe vs. unsafe swallowers) was conducted and sensitivity, specificity, area under the curve (AUC) and likelihood ratios were calculated.

Results: VFSE analysis revealed 24 penetrator/aspirators (PAS >3) and 46 non-penetrator/aspirators (PAS <2). Cough volume acceleration (CVA), peak expiratory flow rise time (PEFRT), and peak expiratory flow rate (PEFR) were significantly different between airway safety groups ($p < 0.05$) and demonstrated significant discriminate ability to detect the presence of penetration/aspiration with AUC values of: 0.85; 0.81; and 0.78 respectively. CVA < 45.28L/s, PEFR <3.97L/s, and PEFRT > 76ms had sensitivities of 91.3%, 82.6% and 73.9% respectively and specificities of 82.2%, 73.9%, and 78.3% for identifying penetrator/aspirators.

Conclusions: Voluntary cough airflow measures identified ALS patients at risk for penetration/aspiration and may be a valuable screening tool with high clinical utility.

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P116 RELATIONSHIP BETWEEN EVALUATION OF SALIVARY VOLUME AND DYSPHAGIA IN PATIENTS WITH MOTOR NEURON DISEASE

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Keywords: saliva, management, deglutition

Background: Excess saliva may interfere negatively in various aspects of life of patients with MND, including: quality of life, quality of sleep, feeding, speech, airway protection and compliance to non-invasive ventilation. The accumulation of saliva in the mouth can be due to an increased production of saliva, generating an increase in volume in the oral cavity or by a difficulty in swallowing of saliva, in which the patient swallows a smaller number of times or has ineffective swallowing. Understanding the process that leads to this discomfort can be the differentiator for management, indicating the most appropriate treatment for each case.

Objective: To assess the amount of saliva produced and the relationship with the occurrence of dysphagia in patients with MND.

Methods: 72 patients were evaluated, 53 (73.61%) with a diagnosis of ALS (26 female, average age 60.84y; 27 male, average age 51.96y) and 19 (26.39%) with diagnosis of PBP (10 female, average age 58.1y; 9 male, average age 62.56y). Speech therapy assessment was made of swallowing, beyond sialometry test, through the technique of cotton weighing. The patient was instructed to swallow the saliva; then six cotton rolls were positioned in the oral cavity, pulled out after two minutes. The patient was asked not to swallow in this period, either feeding or using any substance (such as toothpaste, bullet, cigarette or water) for at least an hour before the exam. When removed, the cotton rolls were again heavy in balance of accuracy, with four decimal digits.

Results: The complaints corresponding to the changes of oral phase were: previous escape; difficulty in grasping; training; maintenance; and food bolus ejection, but stasis in the oral cavity. Pharyngeal phase complaints referred to: stasis in laringopharyngeal region; need for swallowing manoeuvres with effort; and multiple swallowing techniques; occurrence of throat clearing; choking and coughing. As for the sialometry test, patients with ALS showed average 0.638 ml/minute and patients with PBP, average 0.946 ml/minute. Within the group with ALS, 22.64 had PEG and 35.85 made use of NIV; in the group with PBP, 52.6 had PEG and 73.68 made use of NIV.

Discussion and conclusions: Many patients receive medications to decrease the salivary flow. However, it was observed that the group PBP, with greater compromise of the swallowing, showed higher volume of saliva in the oral cavity; demonstrating that there are other factors, such as swallowing and hydration, which can interfere in this increased salivation. This implies the need of assessment of the patient by a multidisciplinary team, so that the conduct must be based for all aspects evaluated by the team, aiming to assist the patient in saliva management.

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P117 PICC LINE AS AN ALTERNATIVE TO GASTROSTOMY IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS AT ADVANCED STAGES

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Keywords: gastrostomy, piccline catheter, tolerance

Background: Gastrostomy is performed in ALS when dysphagia is severe or when weight loss is significant but in some cases it cannot be performed or may raise some concerns, mainly when respiratory insufficiency is severe. Peripherally inserted central venous catheter (Picc line) is a potential alternative for a patient's nutritional support and its use has not been described to date in ALS patients.

Methods: All patients' files from our ALS center since 2012 were reviewed and the outcome of those patients with gastrostomy or with Picc line catheters were compared.

Results: 25 ALS patients with Picc line and 42 with gastrostomy (mainly radiologically inserted) were identified. Picc line was performed because of: failure of gastrostomy procedure (two cases); severe respiratory insufficiency (23 cases). At the time of the procedures, ALS duration was 32 months with Picc line vs 24 for gastrostomy, p = 0.03. Mean delay to death was 4 months for picc line and 9 months for gastrostomy (p = 0.002). In the Picc line group, 2 patients had venous thrombosis and 3 had sepsis. In the gastrostomy group, 23 had at least one complication (peristomal infection, bowel obstruction, diarrhoea, nausea, inadvertent tube removal).

Conclusions: Both procedures appeared safe and no immediate complication was noted. Nethertheless although less severe, complications were more frequent with gastrostomy (23 out of 42, vs 5 out of 25 for Picc line). Delay to death is shorter with Picc line as patients who underwent this technique have a much more advanced stage with severe respiratory insufficiency. In our experience, a Picc line catheter seems a valuable alternative to gastrostomy at advanced stages.

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P118 ARM-BAND EVALUATION OF RESTING ENERGY EXPENDITURE (REE) IN ALS

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Keywords: arm-band, heat dissipation, hypermetabolism

Background: ALS patients (pts) are complex from a nutritional point of view with a progressive decline in lean body mass and motility. At the same time literature shows that 50% of pts are hypermetabolic (REE measured/REE calculated> 1.1) and have progressive dysphagia. As a result, it is important to make a proper evaluation of the resting energy expenditure (REE). Recently a metabolic Holter (Arm Band) is available for use to estimate REEs and total resting energy expenditure (TEE). In clinical practice, equations such as the Harris Benedict equation are used to estimate patient REE.

Objectives: The purpose of this paper is to compare the REEs to the theoretical value calculated with the Harris Benedict equation (REEc).

Methods: Since 2001, the Unit of Dietology and Clinical Nutrition have collaborated with the BENE within the multidisciplinary team, which follows ALS patients. Patients are monitored every 1-3 months for weight, food intake and nutritional indexes. During 2011 in 48 patients, the Arm Band has been placed for a minimum of 3 days to estimate the REEs and the TEEs. REEc was then calculated for each patient.

Results: The 48 patients (mean age 65 years) showed a weight loss between initial weight and weight at the time of arm-band placement equal to 0.2 kg (0.3% despite a range from –20 to +20%). REEs shows values higher than REEc (+11%, range from -4% to +33%). 30 patients (63%) have a REEs / REEc> 1.1, with a REEs average higher by 18%. 18 patients had a REEs/REEc <1.1 with a mean difference of + 3%. The study also showed an inverse correlation between overestimation of REEs and the actual percentage of weight loss. The two groups were matched for age, BMI and sex. No difference was detected in the average temperature of the skin (32.8 vs. 32.9°C), while there was a significant difference in the average amount of heat dissipated (63.8 vs 53.5 W / m2; p < 0, 05).

Discussion and conclusions: Our study shows that the Arm Band provides REEs values similar to those reported in the literature, and that the state of hypermetabolism is somehow connected to impaired heat dissipation.

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P119 EVALUATING THE POTENTIAL OF DIET AND FOOD COMPONENTS AS DISEASE MODIFIERS IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Keywords: nutrition, fatty acids, body mass index

Background: Nutritional management is essential in the treatment of ALS because body mass index and nutritional status are independent, prognostic factors for survival and disease complications. Malnutrition is common in ALS, so that caloric supplementation is recommended worldwide. Recent studies in addition, associate nutrient intake with general ALS risk and recommend monitoring body mass index (BMI) and nutritional state in treatment of ALS. To our knowledge, no study has examined the actual nutrient intake in a population based longitudinal cohort of ALS patients.

Objectives: The first objective of the current study is to unravel the link between nutritional intake and disease status and progress. Secondly, we want to answer the question whether improved outcomes resulting from caloric supplementation are associated with specific nutrients, or simply the provision of excess calories.

Methods: We observe a longitudinal cohort of at least 50 patients over the entire disease course. Nutritional habits via food frequency protocols over 5 days before clinical examination and BMI are obtained every 3 months; clinical status (ALSFRS-R and disease stage) are examined every 3 months; analysis of the fatty acid distribution in erythrocyte lipids, assessment of metabolic parameters like blood lipids and inflammatory markers are analysed every 6 months.

Results: Currently we have included 18 ALS patients (7 females, 11 males; age 62 ± 13 years; BMI 24 ±3 kg/m^2; ALSFRS-R 34 ± 8). Fatty acids in erythrocyte lipids are distributed as follows: saturated fatty acids: 36 ± 2% fatty acid methyl esters (FAME), monounsaturated fatty acids: 22 ± 5% FAME, n-6 polyunsaturated fatty acids: 30 ± 3% FAME; n-3 polyunsaturated fatty acids 8 ± 2% FAME. In comparison with the recommendations of the German Society of Nutrition the dietary habits of patients with ALS are characterized by low intakes of dietary fibers 21 ± 10 g/d, PUFA 11 ± 4 g/d, calcium 677 ± 232 g/d and folic acid 274 ± 115 µg/d. In the further course of the study the collected data are related to disease progress.

Discussion and conclusions: The study allows the collection of substantial data about dietary intake (including energy, fat and fatty acids, carbohydrates, protein and amino acids, vitamins and minerals). The established nutritional protocols in combination with the analysis of the fatty acid distribution in erythrocyte lipids as marker for the intake of fat-rich foods over the last 2-3 months will facilitate a comprehensive insight into the nutritional habits of ALS patients and will help to identify dietary factors that are associated with disease progress or survival.

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Theme 7 Quality of Life and Palliative Care

P120 COPING STRATEGIES USED BY PEOPLE WITH MOTOR NEURONE DISEASE

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Keywords: coping, quality of life, QOL, Multi-centre

Background and objectives: Coping within motor neurone disease (MND) may be considered as the cognitive and behavioural efforts to tolerate the demands of the condition. The aim of our study was to identify coping strategies in MND patients, identifying correlations between coping strategies and demographic differences

Method: As part of the multi-centre, UK TONiC study, MND patients completed the COPE60 questionnaire, and provided data on demographic and clinical characteristics. We generated median frequencies of each coping item, using SPSS22 to compare use between gender, age and disease type using the Mann-Whitney U Test.

Results: We recruited 178 patients; average age of 66 (29-90); 108 males (60.7%); average disease duration 32 months (0-288). Severity was assessed by using ALSFRS-R bulbar, mobility and respiratory subscales with low indicating lower half of the score range for one domain (51.1%), medium for two domains (32.6%) and high three domains (13.5%).

The commonest coping strategies were Acceptance (Md 8) and Active Coping (Md 7); the least common was Substance Use, Religious Coping and Denial (Md 0-1). Those aged <65 used more Focus on and Venting of Emotions (Z=-2.264, p=0.02), Humour (Z=-2.154, p=0.03) and Substance Use (Z=-2.274, p=0.02). Those aged 65 used more Religious Coping (Z=-2.534, p=0.01) and Restraint (Z=-2.334, p=0.02). Women used more Mental Disengagement (Z=-3.004, p=0.003), Focus on and Venting of Emotions (Z=-3.332, p=0.001), Instrumental Social Support (Z=-1.999, p=0.046), Denial (Z=-2.032, p=0.042), Behavioural Disengagement (Z=-2.493, p=0.013), Restraint (Z=-2.289, p=0.022) and Emotional Social Support (Z=-2.046, p=0.041).

Discussion and conclusions: MND patients favour an active, adaptive coping approach. Younger patients seem to use expression of emotion as a way of coping, differing from previous literature. Of concern, we have shown that female patients seem to have a more avoidance patterns of coping, somewhat reflected in other neurological conditions. We have highlighted the resilience of MND patients, whilst identifying that younger patients may benefit from discussion of emotional stressors. Importantly, we have uncovered a trend that female MND patients may be more at risk of maladaptive coping.

Acknowledgements: Holland D, Mills R, Tennant A and Young CA declare no conflicts of interest for this work. The TONiC study received unrestricted grant support from NIHR, the MND Association (UK), Walton Neuroscience Charity, Biogen, Novartis, Roche, Teva

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P121 PSYCHOLOGICAL DISTRESS IN MOTOR NEURONE DISEASE AND ITS CORRELATION WITH USE OF ALCOHOL OR DRUGS AND OTHER NEGATIVE COPING STRATEGIES

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Keywords: coping, HADS, TONiC

Background: Psychological distress in Motor Neurone Disease (MND) has been shown to influence quality of life. Coping strategies may help mediate the effects of psychological distress. However, certain coping strategies have been conceptualised as maladaptive, for example denial. We aimed to identify the prevalence of psychological distress, the effect of age or gender, and any correlation between psychological distress and acknowledged maladaptive coping strategies, such as alcohol/substance use.

Method: As part of the multi-centre, UK TONiC study, MND patients completed the Modified Hospital Anxiety and Depression Scale (HADS) for MND and the COPE60 questionnaire, also providing data on clinical characteristics. Severity was assessed by using ALSFRS-R bulbar, mobility and respiratory subscales, with low severity indicating lower half of the score range for one

domain, medium severity two domains and severe three domains. We calculated HADS scores to identify case frequencies and compared psychological distress by gender and age, as well as the effect of psychological distress on coping strategies, using the Mann-Whitney U Test. All analyses were done in SPSS22.

Results: We recruited 178 patients; 60.7% male; average age 66 years (29-90); average disease duration 32 months (0-288). Severity ranged from 51.1% low, 32.6% medium and 13.5% severe. We identified 26 cases of anxiety (14.8%) and 34 cases of depression (19.2%). Female patients are more likely to suffer from anxiety (X^2=6.490 p=0.011) with a relative risk of 2.5 (95% CI 1.21-5.21); no gender difference existed for depression. There was no significant difference in Anxiety or Depression by age.

Those patients suffering from anxiety tend to use more *Mental Disengagement* (Z=-2.803; p=0.005; r=-0.22); *Venting of Emotions* (Z=-3.977; p=<0.001; r=-0.32); and *Denial* (Z=-2.307; p=0.021; r=-0.19), whereas those not suffering from anxiety use more *Humour* (Z=-3.029; p=0.002; r=-0.25). Those patients suffering from depression are more likely to utilise *Behavioural Disengagement* (Z=-2.609; p=0.009; r=-0.26), whereas those not suffering from Depression tended to use more *Positive Reinterpretation* (Z=-3.975; p=<0.001; r=-0.39); *Social Support* (Z=-2.370; p=0.02; r=-0.23); *Active Coping* (Z=-2.447; p=0.014; r=-0.24); *Humour* (Z=-3.864; p=0.009; r=-0.26); *Acceptance* (Z=-2.098; p=0.036; r=-0.21) and *Planning* (Z=-2.373; p=0.018; r=-0.23).

Conclusion: Anxiety is linked to several maladaptive coping strategies, which highlights the need to address stressors in these patients, particularly in female patients who are more at risk of anxiety. Psychological distress is not associated with alcohol or drug use in MND. Non-depressed patients are more likely to use adaptive coping strategies but these cross sectional data preclude determination of whether treatment of depression is likely to facilitate a different coping profile or whether support of more adaptive coping would reduce the risk of depression. Ongoing longitudinal analyses in TONiC will provide future data.

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P122 THE FATIGUE TO QUALITY OF LIFE RELATIONSHIP IN MND: IS GENDER MODERATING THE EFFECT?

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Keywords: quality of life, fatigue, disability

Background: Fatigue appears to impact upon the physical functioning and perceived quality of life for those with motor neurone disease (MND). Recent advances in analytical techniques provide the opportunity to examine the mediating and moderating effect of variables such as physical disability or gender, upon quality of life.

Objectives: To examine the mediating effect of motor functioning between fatigue and quality of life, and the moderating effect of gender upon this relationship.

Methods: TONiC is an ongoing, multicentre, longitudinal, UK study inviting people with MND to complete a questionnaire pack containing a variety of Patient Reported Outcome Measures (PROMS). Data were analysed on fatigue, using the Neurological Fatigue Index-MND (NFI-MND); disability using the ALSFRS-R Motor subscale; and physical health aspects of quality of life, using the WHOQoL-Bref Physical domain. Data from the PROMS were included in the model as linearised variables derived from Rasch analysis. Age was considered as an independent contextual factor, and gender as a moderator. A Sobel-Goodman mediation test first assessed the mediating effect of activity between fatigue and quality of life, and a path analysis then assessed the invariance of gender for the relationships so identified. A non-significant Chi-Square test of the model was considered a valid result. Post-hoc estimations provided evidence that path coefficients were statistically significant between groups. The analysis was undertaken in STATA13.

Results: Data are analysed here from 178 patients; mean age was 65.6 years (SD 11.0) and mean duration of disease was 2.7 years (SD 3.3). 61.2% were male. The median NFI-MND was 24 (IQR 17.8-28.3); the median ALSFRS-R Motor domain was 10 (IQR 6-14) and the median WHOQoL-Bref Physical was 23 (IQR 19-27). There were no significant differences in the NFI-MND, ALSFRS-R Motor, or WHOQoL-Bref Physical across gender (Mann Whitney p=>0.05).

The Sobel-Goodman mediation test showed a strong partial mediation for ALSFRS-R Motor function, with 28% of the total effect of fatigue upon quality of life mediated through this variable. The basic path model, including age, showed good fit in the path analysis (Chi-Square 1.366; df 1; p=0.242; RMSEA 0.046; CFI 0.998; TLI 0.991). The mediating effect remained at 26% after including the age covariate. There was no evidence that the path coefficients were significantly different between males and females (Chi-Square 7.683; df 6; p=0.262; RMSEA 0.057; CFI 0.992; TLI 0.987).

Conclusion: Fatigue is common in ALS, impacting upon activities, participation and quality of life. This study shows that the effects of fatigue upon quality of life are substantial, both directly, and also indirectly, mediated through motor function. There is no indication that males and females experience this relationship differently.

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P123 EXISTENTIAL LOSS IN THE CONTEXT OF MOTOR NEURONE DISEASE: A HERMENEUTIC PHENOMENOLOGICAL STUDY

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Keywords: existential hermeneutic phenomenology, existential loss, lifeworld approach

Background: Motor Neurone Disease (MND) is a rare, devastating neurodegenerative disease of middle/later life, usually presenting in the sixth and seventh decades (1). People have to wait many months to receive their diagnosis of MND (2), and they have already experienced the degenerative nature that characterises MND (3). However, information on the meaning of life with MND through time is limited.

Methods: The aim was to answer the research question: "What does it mean to be a person living through the illness trajectory of MND?" and to study the phenomenon of existence when given a diagnosis of MND, in the context of receiving health care. Hermeneutic phenomenology inspired by the philosophers Heidegger and Gadamer (4, 5) informs the methodological approach which asks the question: "Can you please tell me the story of your life... since you first thought there might be something wrong with you?" Hermeneutic analysis involved a five-stage process in order to understand (interpret) the lifeworld (6) of four people diagnosed with MND. A lifeworld perspective helps to make sense of the meaning of existence when given a terminal diagnosis of MND.

Results: The concept of 'existential loss' identified in relation to MND was a loss of past ways of being-in-the-world: loss of embodiment, loss of spatiality, and loss of future. The concept of existential loss requires closer attention by health care professionals from the time of diagnosis and through the illness trajectory.

Discussion: The findings are conceptualised into a framework which, used as a clinical tool, may prompt health care professionals to focus on their patient's existential loss and existential concerns. This study adds to the existing literature that is calling for a lifeworld approach to health care.

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P124 THE LOST ART OF KISSING

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Keywords: intimacy, quality of life, clinical team

Background: While ALS may not affect sexual function directly, it can affect self-image, mobility and breathing, which impact sexual activity. Additionally, one's sexual partner often becomes the caregiver, which may further diminish intimacy. Our prior study surveyed ALS clinic team members on perceptions and standard practice regarding intimacy in ALS clinics across the US. Nearly all of those who responded agreed intimacy and sexual relationships are affected by diagnosis and should be discussed. However, only half of those have initiated this conversation with PALS or caregivers, this presents a possible area of need which could be addressed at clinic visits.

Objective: To study the extent to which PALS perceive intimacy to be affected by ALS; their comfort level expressing their concerns and discussing them at clinic; level of satisfaction with support in clinic; and areas of improvement for the clinic team.

Methods: An 11-item online questionnaire was distributed to PALS to assess: perceptions of the effects of ALS on intimacy; their comfort level in intimacy-related discussions at clinic; their history pertaining to such discussions; and with whom and how they desire to discuss this topic, if applicable. Data was anonymously collected over an 8 month period from PALS in the New York area.

Results: At time of submission, 21 completed responses were received from PALS between the ages of 25-74 years with varying degrees of disease severity. 64% of responses were female PALS. Results thus far indicated that 79% of PALS feel ALS diagnosis impacts their intimacy and sexual activity. 60% who were previously sexually active reported decline and change in sexual activity and intimacy. 65% feel that while these issues have not been discussed, they should be addressed by healthcare professionals. Opinions varied on which team member(s) are considered appropriate to discuss these topics but most agreed it is dependent on a patient/caregiver level of comfort. Further results are pending.

Discussion: Evidence suggests that sexuality is a defining element in quality of life and is strongly affected by diagnosis and symptoms of ALS. Due to limited understanding and the sensitive nature of the topic, few studies have been conducted to address this and concerns on intimacy are often avoided by health care practitioners. The aim of this study was to analyze the effect of disease process on intimacy and how well these concerns are addressed by clinic staff. We have designed a similar study for partners, which is currently being distributed to assess the same concerns. Results thus far indicate sexuality and intimacy are affected, patients are open to discussion with qualified clinic staff, but there is a lack of acknowledgement and discussion by clinic team members which might enable better quality of life.

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P125 TEACHING TO TAKE CARE: TRAINING COURSE FOR HOME-BASED CAREGIVERS IN AMYOTROPHIC LATERAL SCLEROSIS: OUTCOMES FROM A PILOT PROJECT

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Keywords: caregivers, healthcare, management of chronic disease

Background: ALS affects the autonomy and functional capacity for self-care of the individual, with a high level of complexity. Patients gradually require specialized assistance from a caregiver to perform all activities of daily living. Care should be provided to patients and their families within a multidimensional support beyond the physical, psychological, spiritual and emotional issues. The lack of specialized resources with paucity of specialized medical centers, leads the families to seek information from sources not always reliable to take care of their family member with ALS. Providing evidence-based orientations can help the caregivers to cope with the challenge.

Objectives: To supply evidence-based skills related to the care of ALS patients at home with safety, and evaluate satisfaction and level of learning with the methodology used.

Methods: The project was carried out during 2013-2014, in the setting of the Portuguese ALS Association, where we developed short-courses with basic themes on safe care at home: What is ALS?; legislation and social support; general care; management therapeutics; technical aids; respiratory assistance at home; caregiver self-care; and emergency situations. The team of instructors was specialized in ALS follow up, and was composed of: Nurse, Social Worker, Physiotherapist and Biomedical Engineer (for augmentative communication devices). The sample included 77 ALS caregivers. We used an expositive and hands-on learning methodology, with written examination with closed questions, and questionnaires of satisfaction applied immediately after the course.

Results: We found an average of 70% correct answers in the written examination, and > 90% of caregiver were very satisfied with the course. These results demonstrate an intensive participation, and successful completion by learners. General health services are not always prepared to follow these patients properly.

Discussion: This project allowed the introduction of a model of management for severe chronic disease which does not overwhelm health services, allowing patients and families to maintain their emotional and social ties in the community monitored by health professionals, and can contribute to avoiding financial and social constraints on ALS patients and their families.

Conclusion: Interventions to meet the needs of caregivers can contribute to improving the quality of life for the caregiver and their family. Future studies should analyze the influence of these types of initiatives on the proper utilization of health care services, which may have impact on hospital re-admissions.

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P126 SEARCH OF FACTORS THAT AFFECT THE POSITIVE PERCEPTION OF ALS CAREGIVERS

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Keywords: home care, family caregiver, positive perception

Objectives: Previous research has demonstrated that caregivers for patients with amyotrophic lateral sclerosis (ALS) can have a positive perception of their caregiving experience. Understanding this positive perception can lead to a more effective nursing approach to mitigate the caregiving burden. The purpose of this study is to explore the factors influencing ALS caregivers' positive perception of caregiving.

Methods: 1) Participants: 1,809 members of the Japan Amyotrophic Lateral Sclerosis Association, which provides homecare to ALS patients; 2) Survey content: ALS patient attributes, caregiver attributes, and 21 items selected from previous research on the factors influencing positive perception of caregiving; 3) Exploratory factor analysis: An exploratory factor analysis was performed on the remaining items using varimax rotation to identify major factors. Cronbach's α was used to test reliability. The extracted factors were named based on item content; 4) The study was conducted with approval from the Kyushu University Internal Review Board for Clinical Research.

Results: Of 866 surveys returned (47.8% response rate), 495 (27.3%) were considered valid. Of the 21 items from the previous research, 6 were eliminated due to ceiling effects or because their item-total correlation coefficients were less than 0.3. The 15 remaining items were considered to be the factors influencing positive perception of caregiving. Exploratory factor analysis yielded a clear optimal solution with a 13-item 3-factor structure. These 3 factors accounted for 32.0%, 11.8%, and 10.1% of the variance, respectively, with a cumulative contribution ratio of 54.1%. Cronbach's α was 0.76 (factor 1), 0.72 (factor 2), 0.78 (factor 3), and 0.81 (total). The factors were named "interaction with the patient," "reinforcement of coping methods," and "support from other caregivers" respectively.

Discussion: Factor 1 (interaction with the patient) consisted of items relating to past and on-going good

relations between the caregiver and the patient, a result that coincided with the findings of a few previous studies. Factor 2 (reinforcement of coping methods) consisted of resources considered to be useful in mitigating the burden of care, such as good relations with support service providers and family cooperation. This factor suggested that caregivers' efforts to mitigate their caregiving burden may influence their positive perception of caregiving. Factor 3 (support from other caregivers) consisted of items related to interaction with other caregivers. This was thought to be a mental factor influencing the positive perception of caregiving, since a sense of personal growth was reported as a result of belonging to a patient association, as the participants in this study did, through providing mutual support, sharing information, and offering advice.

Further research is needed to develop understanding of the relationship of these factors with the caregivers' positive perception of caregiving and their feelings about the caregiving burden for ALS patients.

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P127 PURPOSE IN LIFE AND QUALITY OF LIFE IN ALS PATIENT-CAREGIVER DYADS: A MULTILEVEL LONGITUDINAL ANALYSIS

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Keywords: quality of life, purpose in life, caregiver

Background: Maintaining psychological well-being is a salient goal in providing care to patients with ALS and their caregivers. Quality of life (QOL) has been an important focus in the literature. However, other positive psychological aspects have received less attention. Previous research suggests that patients maintain QOL and exhibit psychological resiliency. In contrast, caregivers experience decreases in QOL. Previous work suggests that existential aspects of QOL may be stable sources of well-being for both patients and caregivers. Purpose in life (PIL) is an existential construct thought to be independent of adversity. PIL associates with positive psychological indices (eg, mood and happiness) and markers of better physical health spanning from biomarkers (eg, cardiovascular and immune) to decreased risk of premature mortality. Previous research reported that PIL attenuated the relationship between physical brain pathology and cognitive ability in patients with Alzheimer's disease.

Objective: This study aimed to examine the stability of PIL and QOL in relation to diagnosis and end of life in patients with ALS and their caregivers.

Methods: The study sample included 130 patients with ALS (66% male, Mean age = 61) and 110 caregivers (28% male, Mean age = 57) from the Seattle ALS Patient Profile Project. PIL and QOL were assessed in the patients' homes 7 times, over the course of 18 months. Trajectories were evaluated through multilevel modelling.

Results: The proportion of total variance in PIL attributed to stable individual differences was 75% for patients and 78% for caregivers, reflecting substantial stability over time. Variance in QOL attributed to stable individual differences was lower than for PIL: 59% for patients and 57% for caregivers. Periods of decline and stabilization were observed in PIL and QOL. Growth models revealed a moderation pattern in which individuals who enrolled closest to diagnosis (Patient PIL proximity to dx: $p<0.001$; Caregiver PIL: $p=0.006$; Patient QOL: $p=0.008$; Caregiver QOL: $p=0.06$) and closest to patient date of death (Patient PIL proximity to death: $p=0.01$; Caregiver PIL: $p=0.003$; Patient QOL: $p=0.05$; Caregiver QOL: $p=0.02$) both showed the fastest decrease in PIL and QOL over time, though some results did not reach statistical significance. In contrast, individuals enrolled at the middle phase of their disease showed less decline in PIL and QOL.

Conclusion: PIL was more stable than QOL and therefore a potential psychological resource for both patients and caregivers. Therapies aimed to foster meaning and purpose may benefit patients with ALS and their caregivers. Furthermore, critical periods exist (ie, following diagnosis and approaching death) in which intervention may be needed most. Applying early psychological intervention, following patient diagnosis, may potentially mitigate future decline in well-being.

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P128 THE EXPERIENCE OF BURDEN OVER TIME IN FRONTOTEMPORAL DEMENTIA AND MOTOR NEURON DISEASE

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Keywords: carer burden, longitudinal study, FTD-MND

Background: Frontotemporal dementia (FTD) and motor neuron disease (MND) fall within the same disease spectrum. Carer burden in FTD subtypes (behavioural-variant FTD and semantic dementia) has not yet been directly compared with that of patients who also have the adjunct diagnosis of MND (FTD-MND). The evolution of burden with disease progression may also differ across these syndromes.

Objectives: To examine carer burden longitudinally in three patient cohorts: bvFTD, SD and FTD-MND.

Methods: Perceived carer burden was evaluated using the Zarit Burden Interview (ZBI) in patients with bvFTD (n=21), svPPA (n=18) and FTD-MND (n=15) at the initial clinical presentation and follow-up assessments. The Mini-Addenbrooke's Cognitive Examination (M-ACE) and the Motor Neuron Disease Behaviour Scale (MiND-B) were also used. Linear mixed effects models examined longitudinal changes on the ZBI, M-ACE and MiND-B across groups.

Results: Burden at baseline was highest for the bvFTD group. Longitudinally, perceived burden increased for the SD and FTD-MND groups whereas in bvFTD, the level of burden was high at baseline and remained high with disease progression. Significant time and diagnosis interactions were not obtained for the M-ACE or MiND-B. The severity of abnormal behaviours (MiND-B) at baseline significantly correlated with baseline levels of carer burden and furthermore, accounted for almost a quarter (23%) of the variance in carer burden at the first clinical follow-up.

Discussion and conclusions: The trajectory of perceived burden differs across bvFTD, SD and FTD-MND. Levels of burden increase for SD and FTD-MND whereas they remains high for bvFTD carers. The evolution of burden in these three syndromes may be a reflection of the way in which these syndromes are clinically characterized for the carer. Moreover, psycho-education programs for carers which provide better coping strategies for challenging behaviours may reduce levels of burden experienced with disease progression.

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P129 CAREGIVER BURDEN IN ALS – DIMENSIONS AND DIFFICULTIES

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Keywords: caregiver burden, dimensions, themes

Background: Management of ALS is palliative, aimed at maximising quality of life and minimising the burden of disease for patients and caregivers. Caregiver burden is a broad term and involves difficulties associated with assuming the caregiving role and operating in that role, and physical, psychological and emotional well-being.

Objectives: To describe the burden and difficulties experienced by informal caregivers of people with ALS in Ireland.

Methods: The analysis is part of a longitudinal study of 100 people with ALS, and their primary informal caregiver recruited from those attending a multidisciplinary clinic in Dublin. Information is collected on a range of demographic and socio-economic factors, and standardised measures assess depression and anxiety, quality of life and caregiver burden, as part of a semi-structured interview with caregivers in their home. In addition respondents are asked to outline what for them, are some of the difficult things about caregiving. Descriptive statistical and inductive thematic analysis, provide a comprehensive picture of burden and difficulties associated with informal caregiving in ALS.

Results: The analysis is based on 78 caregivers at their baseline interview. This group is predominantly female; a majority are spouses/partners of ALS patients, with an average age of 55 years. The mean burden score from the Zarit Burden Interview (ZBI) is 27.4, and 53% are in the high burden category (1). Caregivers were asked about some difficult aspects of caregiving and a number of interrelated themes were developed from the responses given: the practicalities of the ALS condition; impact on the psychosocial and emotional wellbeing of the caregiver; limitations and restrictions; the effect on relationships.

Discussion and conclusion: The respondents indicated a moderate level of quality of life. There is an indication of anxiety in the normal range and mild depression, with a majority of caregivers already in the high burden category. A global burden score may not adequately capture the experiences of burden, and the dimensionality of that burden is explored. Themes reflect the general dimensions of burden discussed in the literature, as difficulties cluster around taking on the caregiver role and functioning in that, and the impact on the caregivers own psychosocial and emotional wellbeing. Caregiver's physical, mental and emotional health influences their ability to provide care to patients with ALS. It is important to be supportive of the care needs of caregivers.

Acknowledgements: The project is funded by the Health Research Board (HRB) Dublin as part of the HRB Interdisciplinary Capacity Enhancement Awards.

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P130 CLINICAL RESEARCH CONTINUUM: TRANSPARENCY, PRIVACY AND PATIENT-CENTRICITY

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Keywords: patient-centricity, research continuum, NeuroBANK™

Background: People-Powered ALS Research Networks as promoter of patient-centric research in ALS will unify and empower PALS, caregivers and their affinity groups to move beyond conventional research participation, advocacy, and fundraising efforts to improve care for and ultimately eradicate the disease. Through direct participation in decisions on research and drug development, patients influence research priorities and directions.

Objectives: To develop a collaborative network that represent an all-inclusive model to improve care and drug discovery for ALS.

Methods: Utilization of a single research environment, which includes: development of consent from researchers and clinicians on Common Data Elements (CDEs); best practices and standards for information collection during patient clinical and research visits (including data, biospecimens, images and genetic files), and shared patient-reported outcomes (PROs); scales; questionnaires and trackers available via patient portals to provide tools accessible to all stakeholders. Adoption of the patient Global Unique Identifier technology - GUID - for PALS population making it possible to share information aggregated from multiple sources and locations, while preserving PALS' privacy.

Results: The implemented accelerated research environment with information central repository - NeuroBANK™ - allows for efficacies in research and care. Information acquisition is promoted and encouraged through the entire patient clinical lifecycle. GUID technology allows for a longitudinal view on patient information, regardless of the location where it was generated or captured. Patient-entered information may be merged with data in NeuroBANK™. The introduction of common standards and nomenclature for biospecimen collection promotes sharing of these finite resources. A centralized search facility allows researchers to query clinical and phenotypical data, and discover the availability of corresponding biospecimens and genetic files. The collaboration with EHR vendors like Epic and Cerner promotes standards for data collection in clinics and allows for data exchange with the central repository. An approved "umbrella" research protocol and consent language allows consenting PALS to be recruited for clinical data capture and sharing.

Conclusions: Building such an ALS Clinical Research Continuum is not for the weak at heart. Multiple obstacles will need to be overcome, with regulatory and ethical challenges moving to the forefront. While the current post-Ice Bucket Challenge environment is encouraging, development of an even more supportive research ecosystem and healthier incentives for collaborations is a must as it is lagging behind. Harmonization of regulatory and ethical requirements for clinical and research information sharing (including data, biospecimens, images and genetic files), the development of common consent language and unique patient identification will strengthen further an international collaboration.

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P131 RESEARCH PRIORITIES IN PALLIATIVE AND END OF LIFE CARE: MND RESULTS FROM A BROAD ANALYSIS OF PATIENT VIEWS

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Keywords: palliative care, research priorities, patient views

Background: There can be a mismatch between what the public see as important unanswered research questions and those which are actually studied. The Palliative and end of life care Priority Setting Partnership (PeolcPSP), facilitated by the James Lind Alliance, identified and prioritised questions about palliative and end of life care that people in the last years of life, current/bereaved carers and professionals feel are important for research to address.

Methods: A national public survey was designed to reveal the question(s) patients, carers and health and social care professionals have about care, support and treatment for people who are in the last few years of their lives. The online survey was widely promoted to the palliative care and MND community in the UK and Ireland. Data were

subsequently uploaded into NVivo 10 (qualitative analysis software) for coding.

The data were analysed using an inductively derived coding framework to draw out research questions. Patients, carers and professionals were then involved in prioritising these research questions.

Results: From the 1,403 responses received from the initial survey, 749 research questions were identified. The focus of the analysis was to identify 'intervention' research questions, where the patient group, the treatment or intervention and outcomes were clearly defined in the question – also known as 'PICO' analysis (Population Intervention Comparison and Outcome). Questions that did not fit this were considered out of scope.

Questions answered by systematic review, any duplicates and those out of scope were removed. The long list of 83 research questions were categorised into five themes: 'Communication'; 'Managing symptoms and medications'; 'Support'; 'Service use'; and 'Understanding dying', and were presented for prioritisation online. A final priority setting workshop, involving patients, carers and professionals then took place to identify the top 10 research priorities (1).

Within the 83 questions, seven specifically raised palliative and end of life care for people with MND/ALS. Five of these were about managing the symptoms of the disease, one on the place and type of palliative care and one in the 'understanding dying' theme.

A secondary thematic analysis of the dataset looked at all of the 65 responses submitted by people responding about MND/ALS. Using the same coding framework, these responses revealed concerns about access to, and coordination of, services; the appropriate introduction of palliative care; and symptom management and care at end of life.

Conclusions: The PeolcPSP report and secondary analysis from the dataset provides evidence of possible directions for future palliative and end of life care research – identified by those most affected.

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P132 TRANSITIONAL CARE FOR 100 PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: transitional care, quality of life, training

Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder. ALS patients suffer from poor life quality and mostly die from respiratory failure. Transitional care can improve the life quality of ALS patients, significantly reducing the risk of readmission.

Objectives: In the present study we investigated the effect of transitional care on enhancing the life quality of ALS outpatients.

Methods: A survey study which included 100 ALS patients was conducted. In the survey Amyotrophic Lateral Sclerosis Functional Assessment Scale (ALSFRS-R), Pittsburgh sleep quality index (PSQI), Self-Rating Depression Scale (SDS), and Self-Rating Anxiety Scale (SAS) were used to assess the effect of transitional care on improving the life quality. The procedures of transitional care include (1) basic training such as sunbathing, soaking arms and feet in warm water, physical rehabilitation exercise, functional training on respiratory muscles (pursed lip breathing and abdominal breathing), and back percussion-induced sputum elimination; (2) nutritional support; and (3) psychological support. Additional transitional care would go to the patients with the onset of the medulla such as lip resistance training, strength and movement training on tongue and soft palate, and exercises of pronunciation and vital capacity singing. Follow-ups were carried out via the telephone, home visit, connection with WeChat, and regular meetings with patients.

Results: ALSFRS-R is a reliable assessment to evaluate the life quality of ALS patients. There were no complications found in all the 100 ALS patients who received transitional care. PSQI showed that 1% of the population had the trouble with dyssomnia before receiving transitional care whereas no patients had dyssomnia after receiving transitional care for half a year. SAS and SDS reported that anxiety and depression were found in 3% and 16% of the population respectively before transitional care. After treated with the transitional care for half a year, the population who suffered from anxiety and depression was decreased to 0% and 9% respectively.

Discussion and conclusions: The daily life function, psychological status and sleep quality of ALS patients

were markedly improved after receiving transitional care. Meanwhile the transitional care also greatly contributes to improving caregiver's competence.

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P133 THE ROLE OF PALLIATIVE CARE IN A MULTI-DISCIPLINARY ALS CLINIC

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Keywords: palliative care, quality of life, multidisciplinary care

Objective: Last year we designed a study to determine how comfortable patients and their caregivers are with discussing future directives with clinic staff and to assess the level of knowledge that clinicians have about palliative care, and ask about their comfort levels in having palliative care discussions with terminally ill patients. Based on this data we are now exploring the need for palliative care knowledge for each role in a multi-disciplinary clinic, and if a palliative care specialist is necessary for ALS clinics.

Background: ALS is a progressive terminal neurodegenerative disease that follows a relatively predictable clinical trajectory, as such palliative care consultation should begin soon after diagnosis. In 2010 in a randomized trial the introduction of palliative care early after diagnosis for patients with metastatic non-small-cell lung cancer increased median survival and had better quality of life than patients assigned to standard care. However, currently clinicians and patients tend to avoid palliative care discussions until the final stages of disease progression and deterioration largely due to the stigmas associated with palliative care.

Methods: The study population includes patients with ALS, their caregivers, and the ALS multidisciplinary clinic team. A questionnaire containing 10 questions was administered to patients and their caregivers during clinic to assess their comfort in addressing future concerns with the clinic team. A separate questionnaire containing nine questions was given to clinic staff to assess the level of general understanding of palliative care and its treatment goals. We analyzed the patient responses to characterize their general experiences in our clinic, and accounted for the clinicians responses of their general knowledge level of palliative care.

Results: We received a total of 21 responses from patients/caregivers. Respondents answered positively (agree/strongly agree) when asked if they like to be prepared for future events before they happen. There was no statistical significance for whom patients preferred to have discussions with about future planning and directives. There were a total of 8 respondents for the Clinician survey, 50% answered they were comfortable discussing future plans and directives with patients, 38% reported being very comfortable, and 12% said it depends on the patient. All respondents answered that they had a clear understanding of palliative care, however only 30% of them could provide the correct definition of palliative care.

Conclusions: Because complex illnesses such as ALS, thrusts patients into a decentralized and confusing healthcare system, palliative care seeks help from many sources typically found in multidisciplinary clinics. By educating clinicians on palliative care and seeking answers from patients and their caregivers about future planning, we hope to discover when patients want to begin discussions about advanced directives and treatment goals throughout their disease in an open forum established in clinic.

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P134 DEATH WITH DIGNITY IN WASHINGTON AND OREGON PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: end-of-life, hospice, care

Background: The Death with Dignity (DWD) Act went into effect in 1997 in Oregon and 2009 in Washington. Since then, 1876 terminally ill patients have sought medications to end their lives. Of these patients, amyotrophic lateral sclerosis (ALS) is the most represented neurological disease.

Methods: We compared institutional-level data across three tertiary medical centers in the Seattle/Puget Sound region to publicly available data from the states of Washington and Oregon.

Results: We identified 39 ALS patients from Washington who participated in DWD. Of the 39 patients, 92% have since died with 81% using the prescribed medication. The median age of symptom onset was 64 (range 42-83). In Oregon, 100 patients sought a prescription with 80% using the medication. The median age of death was 71 years (range 34-86). In both states, the major patient

reasons for requesting DWD reported by physician participants were loss of autonomy and dignity and decrease in enjoyable activities. Inadequate pain control, financial cost, and loss of control over their body were the least important factors. In both states, more patients with ALS were non-Hispanic white, married, educated, enrolled in hospice, and died at home than the general DWD population.

Conclusions: Participation in the DWD Act in Oregon and Washington is a rare occurrence in patients with ALS. For those choosing to participate, strong motivators include a loss of personal autonomy and ability to engage in pleasurable activities rather than pain or cost of care.

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Theme 8 Epidemiology

P135 UPDATE ON THE UNITED STATES NATIONAL AMYOTROPHIC LATERAL SCLEROSIS (ALS) REGISTRY

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Keywords: national, registry

Background: In October 2010, the federal Agency for Toxic Substances and Disease Registry (ATSDR) launched the National ALS Registry to collect/analyze data regarding persons with ALS (PALS). The main goals of the Registry are to determine the incidence and prevalence, characterize the demographics, and examine the potential risk factors for PALS in the United States. Since being launched, the Registry has undertaken enhancements that will help advance our overall knowledge about the disease, aetiology, and make this a comprehensive Registry for both PALS and researchers alike. Articles on ALS national prevalence and risk factors have provided a better estimate of cases and insight to potential aetiologies, respectively.

Objective: Describe completed enhancements to the United States National Amyotrophic Lateral Sclerosis (ALS) Registry.

Methods: ATSDR has implemented several new initiatives including integrating state and metropolitan area-based ALS surveillance projects; deploying a research notification system; launching an ALS biorepository; publishing articles on risk factors and a first-ever national estimate of ALS prevalence. The state and metropolitan area-based ALS surveillance projects are helping ATSDR test the completeness of the Registry. The Registry's research notification system is allowing Registry-enrolled PALS the opportunity to directly link with researchers across the country who are conducting clinical trials and epidemiologic studies. A biorepository was launched in the fall of 2015 to collect specimens for future analysis and examination.

Results: The state and metropolitan area-based surveillance efforts have resulted in increased focus, outreach, and enrollments of patients living in these areas, and have provided more accurate estimates of the incidence and prevalence rates of PALS in 11 distinct geographic areas. The research notification system has resulted in the notification of over 40,000 Registry-enrolled PALS about clinical trials and research studies that have the potential for advancing knowledge on medical treatments. The collection of bio-specimens from PALS will allow researchers to determine potential genetic markers for ALS as well as facilitate the analysis of epidemiological data to assess possible etiologies as well as viable treatment options. In 2011, over 12,000 cases of ALS were found in the United States with a prevalence of 3.9/100,000. ALS risk factors such as smoking, drinking, occupation, and military history were consistent with other published studies.

Conclusions: The National ALS Registry continues to implement enhancements in order to expand ALS research initiatives and public health knowledge about this disease.

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P136 NATIONAL AMYOTROPHIC LATERAL SCLEROSIS (ALS) BIOREPOSITORY FEASIBILITY STUDY

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Keywords: biorepository, registry, specimens

Background: The Agency for Toxic Substances and Disease Registry (ATSDR) launched the congressionally mandated National ALS Registry to identify ALS cases in the United States. To enhance the Registry, ATSDR is coordinating a feasibility study to find the best way to collect and store biological samples for future ALS related research.

Objective: Determine the feasibility of developing a biorepository linked to the National ALS Registry.

Methods: An expert panel meeting in March 2012 provided input into the types of research that could be done to advance ALS research and the specimens needed. The pilot study included two specimen collection components: 1) in-home collection of blood, urine, hair, and nail, on two occasions approximately six months apart; and 2) postmortem collection of brain, spinal cord, CSF, bone, muscle, and skin. Those who participated in the postmortem part of the study collection could also take part in the in-home component. Specimens were processed into smaller aliquots for future distribution. Brain and spinal cord were processed into fixed and frozen sections. The combination of these components allows a thorough evaluation of the challenges associated with assembling a national, population-based biorepository. Eligible participants for either component of this study must have enrolled in the Registry (CDC IRB protocol #5768) and consented to be contacted about research projects.

Results: Three hundred and thirty-nine people consented to donate biological specimens and 330 provided specimens at least once. About 25% did not complete the second draw mostly due to death and illness. There was at least one participant from each state. The age distribution of participants ranged from 31 to 87 years and 60% were male. Fifty-four percent of participants lived 50 or more miles from an ALS specialty/referral center. Thirty people

consented to postmortem tissue donation from 18 states. To date, 11 donations have been completed. All of the participants in the postmortem portion of the study also provided specimens for the in-home collection. The age range of postmortem participants was 43 to 75 years and 50% were male.

Discussion and conclusions: Creating a geographically diverse biorepository has unique challenges. Recruitment was slower than expected, finding reliable phlebotomists across the country was difficult, and there were unexpected logistical issues related to shipping specimens including higher than average temperatures and mechanical failure. However, with some modifications to the pilot project, it is feasible to incorporate a biorepository into the National ALS Registry. These specimens will be a valuable resource for researchers who will be able to request specimens for future ALS studies.

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P137 NATIONAL AMYOTROPHIC LATERAL SCLEROSIS (ALS) REGISTRY: A MODEL FOR RECRUITING PATIENTS FOR RESEARCH, CLINICAL TRIALS, AND EPIDEMIOLOGICAL STUDIES

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Keywords: clinical, trials, recruitment

Background: Researchers face challenges in subject recruitment, especially for rare, fatal diseases like ALS. These challenges include having sufficient sample size as well as meeting eligibility requirements such as age, sex, and study proximity. Similarly, people living with ALS (PALS) face difficulty finding and enrolling in research studies for which they are eligible.

Objective: To describe how the federal Agency for Toxic Substances and Disease Registry's (ATSDR) National ALS Registry is linking PALS to scientists who are conducting research, clinical trials, and epidemiological studies.

Methods: Through the Registry's online Research Notification Mechanism, PALS can elect to be notified about new research opportunities. The Mechanism allows researchers to upload a standardized application with study goals and objectives, along with proof of Institutional Review Board (IRB) approval. If the application is approved, ATSDR queries the Registry for PALS meeting the study's specific eligibility criteria, and then distributes the researcher's study material and contact information to PALS via email. PALS have to directly contact the researcher to take part in any research. Such an approach allows ATSDR to protect the confidentiality of Registry enrollees.

Results: Since the Research Notification Mechanism's deployment in May 2012, approximately 96% of Registry enrollees have elected to be notified about new ALS research opportunities. To date, a total of thirteen institutions around the U.S. have leveraged this tool and over 45,000 emails have been sent to PALS in all 50 states.

Conclusions: The National ALS Registry's Research Notification Mechanism is an effective tool for connecting PALS with ALS researchers. It also benefits researchers by helping to speed-up the recruitment process, increasing the study sample size, and easily and efficiently identifying PALS meeting specific eligibility requirements. As more researchers learn about and use this Mechanism, both PALS and researchers can potentially advance treatment strategies for ALS.

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P138 INCIDENCE OF ALS IN BRITISH COLUMBIA, CANADA: A 5-YEAR RETROSPECTIVE STUDY

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Keywords: health economics, burden of disease, disease trends

Background: A systematic review of ALS in Canada revealed only six studies had been published on disease burden between 1974-2004, none of which were from British Columbia (BC) (1). Reporting provincial population data provides necessary baseline values for future epidemiological studies to properly understand disease trends over time. Additionally, the increasing disability associated with ALS makes characterizing the burden of disease useful for future allocation of health care resources. There is a clear need to examine the burden of ALS in BC more robustly.

Objectives: 1) To determine the five-year crude incidence rates of ALS from 2010 through 2014 in BC; 2) To better characterize the demographic patterns of disease in this population.

Methods: This is a 5-year retrospective study including patients diagnosed with ALS from January 2010 through December 2014. Data was extracted from the ALS Centre at the GF Strong Rehabilitation Centre in Vancouver, BC and then recaptured from the ALS Society of BC patient database. Data extracted included date of diagnosis, age at diagnosis, and sex for patients with El Escorial criteria of definite or probable ALS. All rates obtained were capture-recapture estimations.

Results: From 2010 through 2014 there were 388 overlapping cases identified among both sources, 129 unique to GF Strong, and 173 unique to the ALS Society.

Cumulative incidence was 3.29 cases/100,000 (CI 95% 3.05 – 3.53). Annual incidence peaked at 3.77 cases/100,000 (CI 95% 3.21 – 4.33) in 2013. Males represented 52% of new cases during the 5 years at 3.50/100,000 (CI 95% 3.17 – 3.85) and the greatest number of new cases arose in patients between the ages of 70 – 79 at 14.27/100,000 (CI 95% 12.36 – 16.19).

Discussion and conclusions: Incidence rates in BC are consistent with international figures (2). The authors support the use of capture-recapture analysis for more complete case ascertainment and to account for cases missed by individual sources. Speculation is often made regarding shifting patterns of ALS, and so these findings provide a foundation for subsequent studies to refer to. Additionally, though many confounders complicate annual spending for health resource providers, better understanding of case numbers may better explain expenditure patterns. Future work includes examining the incremental effects new patients have on cost burdens faced by local care providers.

Acknowledgements: Thank you to the ALS Society of BC, the ALS Centre team members at GF Strong Rehabilitation Facility especially Eva Cham, and the Centre for Health Evaluation & Outcome Services (CHEOS) especially Joseph Puyat.

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P139 DATA-MINING IN PRO-ACT: DIAMONDS IN THE ROUGH

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Keywords: PRO-ACT, data sharing, collaboration

Background: The Pooled Resource Open-access ALS Clinical Trials (PRO-ACT) platform and database houses the largest harmonized dataset from 17 completed clinical trials in ALS (8,635 subjects). The initial goal of PRO-ACT was: to establish a common research support infrastructure; to merge and integrate patient data from completed ALS clinical trials; to create a powerful new open-access research tool for researchers, statisticians, clinicians, and anyone interested in "Big Data". With more data coming into PRO-ACT, it is essential to create a data analytical support environment, in which those clinicians/investigators who have neither the time, the statistical skills nor the desire to analyze data themselves but are excellent in asking the right questions, may do so in a confidential and trusting manner. Such support would encourage establishment of a collaborative network, in which not only raw data but results and analytical methods may be published and shared.

Methods: There were close to two million records and more than eight million individual values in the initial PRO-ACT dataset that was released in 2013. Phase II dataset adds data from six new trials, including two highly-anticipated datasets from dexpramipexole and Ceftriaxone trials, and brings the number of records in the combined dataset to more than 2,850,000 from 10,724 subjects. The new data were analyzed, went through rigorous quality assurance and cleaning, and harmonized with original Common Data Elements (CDEs) developed earlier. Adverse Events and Concomitant Medications records were re-coded to the industry standard data dictionaries, MedDRA and WHO-Drug correspondingly.

Results: The updated database contains the largest in the world ALS-related dataset, including such CDEs as demographics, vital capacity, ALSFRS(-R), vital signs and ALS history. While there are more than 500 registered users who downloaded the data, and tens of papers with PRO-ACT-based research are published, the Data Analytics group at the Neurological Clinical Research Institute gets a constant stream of requests from ALS clinicians and researchers asking for help in data analyses and modeling. These requests could be put into three major categories: medications/supplements-related; disease-staging-related and outcomes-related. For instance, research on Vitamin D in ALS and reversible ALSFRS was published recently.

Conclusions: While there is an enormous interest in PRO-ACT data, we still consider this dataset to be underutilized. Ease of use or lack of biostatistical knowledge could be the constraining factors. Organization of the Data Analytics group at the NCRI and similar efforts will increase number of analyses while encouraging national and international collaborations. Harmonization of regulatory and ethical requirements for clinical and research information sharing (including models, methods and secondary data derived from analyses), development of PRO-ACT Commons and its policies that would promote, protect and reward secondary data sharing and acknowledgment of data submitters who share data as co-authors, will further strengthen an international collaboration.

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P140 CLINICAL PROFILE OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS FOLLOWED AT REFERRAL CENTERS IN MINAS GERAIS/BRAZIL

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Keywords: clinical aspects, epidemiology, Brazil

Background: Despite its severity, there are few clinical-epidemiological studies of amyotrophic lateral sclerosis (ALS) in Brazil.

Objectives: To describe the first series of cases of amyotrophic lateral sclerosis (ALS) in Minas Gerais (Southwest of Brazil) and to verify possible associations between neuropsychiatric symptoms (anxiety and depression) and clinical aspects of the disease.

Methods: This is a cross-sectional and descriptive study of a consecutive series of patients with probable or defined sporadic ALS according to Awaji's criteria, followed at two referral centers of Belo Horizonte: Hospital das Clínicas/UFMG and at Hospital Júlia Kubitschek/FHEMIG. Patients underwent clinical, functional and psychiatric (anxiety and depression questionnaires) assessment. Comparisons of patient subgroups were made and correlations between clinical features, functional and psychiatric parameters were investigated.

Results: Sixty-one patients were included, with 38 cases (62.3%) being male, resulting in a male/female ratio of 1.6:1. The mean age at onset of symptoms was 54.9 years (SD 11.4). The mean age at diagnosis was 56.3 years (SD 11.1) and the average time to obtain the diagnosis was 1.7 years (SD 2.0). The average age at assessment was 58.6 years (SD 10.7). Regarding the initial form of presentation, six cases (9.8%) were bulbar, 43 cases (70.5%) were spinal and 12 cases (19.7%) were generalized. Anxiety was found in 18 patients (29.5%) and depression in 20 patients (32.8%). When comparing patients with and without depression, there was significant difference regarding symptoms of anxiety ($p < 0.001$) and there was no difference in relation to disease duration or on functional scale score. A positive correlation between anxiety and depressive symptoms was found ($p < 0.001$).

Discussion and conclusion: The observed results are globally similar to other national and international series, but we found a smaller amount of patients with bulbar symptoms as the initial form of presentation. The characterization of the profile of ALS patients is the first step to improve the quality of the medical assistance and to provide essential data for further studies.

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P141 MOTOR NEURON DISEASE POST POLIOMYELITIS SYNDROME: ANTHROPOMETRIC PROFILE IN BRAZILIAN PATIENTS

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Keywords: anthropometry, weight gain, post-polio syndrome

Background: Post-polio syndrome (PPS) is a disease of the lower motor neuron. Causes of PPS are not fully understood, but the cause more accepted worldwide is the dysfunction of giant motor units formed after acute polio. PPS is characterized mainly by new muscle weakness, with or without muscle fatigue and muscle pain. Evolution is slow progressive, as well described in the literature and can compromise the nutritional status of patients.

Objectives: To evaluate the anthropometric profile in out patients with MND/PPS.

Methods: We collected data of 126 patients diagnosed as MND/PPS. To describe the anthropometric profile we collected the following information: age; sex; weight; height (BMI); age of acute polio; age of the onset of of new muscle weakness; weight gain the period of five years preceding the interview; dysphagia symptoms; constipation and residual sequel that the patient presented.

Results: Mean age was 48.3 years 7.0 (range 31 to 65 years), 81 female (64.2%) and 45 male (35.7%). Mean age on acute polio was 1.9 years 1.7 (range 15 days to 7 years). The residual sequelae of poliomyelitis: monoaresis 73 patients (57.9%), diparesis 41 (32.5%), triparesia 6 (4.7%) and tetraparesis 6 (4.7%). Mean age onset of the new muscular weakness was 40.2 7 years. Body mass index (BMI= weight (Kg)/height (m)) was evaluated according to WHO classification (1998), we observed 52 patients (41.3%) overweight, 6 (4.8%) II obesity, 17 (13.5%) I obesity, 48 (38.2%) eutrophic, 1 (0.8%) Low moderate weight and 2 (1.6%) Low light weight. Weight gain was reported for 70 (55.5%) patients; the average weight gain was 10 kg 7.65Kg. Dysphagia was reported by 35 patients (28%) and constipation 37 (29.4%).

Discussion and conclusions: Most patients 75 (59.5%) were classified as obese; everyone had mobility with preserved gait, and the majority (90.4%) had sequelae of monoparesis and diparesis. The characteristic of poliomyelitis is similar to developing countries affecting children <2 years of age, where, most polio survivors are less severe cases, however, the form of disease progression and symptom severity of PPS can influence decrease in energy expenditure beyond mobility, facilitating weight gain. Orientation and nutritional follow-up is essential to assist the patient in the most appropriate food choices for health promotion, to ensure better conditions for mobility and quality of life.

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P142 MOTOR NEURON DISEASE/ SEUQUELAE OF POLIOMYELITIS AND POST-POLIOMYELITIS SYNDROME DIAGNOSES IN A TERTIARY CENTER

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Keywords: Post-polio syndrome, clinical evaluation, diagnoses

Background: Post-polio syndrome (PPS) is a disease of the lower motor neuron that affects polio survivors years after recovery from an initial acute attack of the poliomyelitis virus. Most often, polio survivors start to experience new gradual weakening in muscles that were previously affected by the polio infection. The most common symptoms include slowly progressive muscle weakness, fatigue (both generalized and muscular), mulcular and articular pain, and a gradual decrease in muscles size (muscle atrophy). The diagnosis of PPS relies entirely on clinical information based on criteria diagnosing PPS.

Objectives: To describe the confirmed diagnostic hypothesis in patients with polio sequelae attending in a specific SPP clinic at UNIFESP/EPM neuromuscular center.

Methods: An interdisciplinary team prospectively evaluated 791 patients according to SPP diagnostic criteria. The evaluation consisted of two steps: 1. Directed interview with a specific SPP protocol and request of laboratory tests, electromyography, electrocardiogram, polysomnography, magnetic resonance imaging and muscle biopsy when necessary. 2. Clinical evaluation, analysis of exams and diagnostic confirmation. For data analysis, we used the software SPSS. Comparisons of parametric variables were obtained by Student's t test.

Results: Mean age was 46 years 9.7 (range 21 to 79 years), 456 females (57.6%) and 335 males (42.4%). Mean age on acute polio was 1.4 years 2.0 (ranging from 3 days to 30 years). 246 (31.1%) patients were diagnosed as having Poliomyelitis sequelae (ICD10 B91), 446 (52.4%) as Post-Polio syndrome (ICD10 G14). The average plateau of stable neuromuscular function was 37 years. Eighty five patients (10.7%, 62 females and 23 males) presented new muscle weakness but did not fully meet the diagnostic criteria. The associated diseases were diagnosed: Encephalic diseases: Stroke 8.2%; Parkinson Disease 2.3%; Multiple Sclerosis 3.5%; unspecific encephalopathy 1.2%; spinocerebellar ataxia 1.2%. Myelophaties: Myelophaty/discophaty 15.3%; HTL V1 1.2%. Degenerative disc disease: discophaty 2.3%. Neurophaties: Chronic inflammatory demyelinating polyneuropathy 1.2%; Charcot-Marie-Tooth 1.2%. Neuromuscular junction disease: myasthenia gravis 5.9%. Myophaties: Metabolic myophaty 1.2%; Myotony 1.2%; Sjögren syndrome 1.2%. Sistemic Diseases: systemic lupus erythematosus 3.5%; diabetes mellitus 15.3%; liver disease 3.5%; hypothyroidism 30.6%. Fourteen patients (1.8%) had ongoing investigation.

Discussion and conclusion: As there are no tests to definitively confirm PPS, this condition may be difficult to diagnose. The interference of other medical conditions in the evaluation must also be taken into account. 10.7% of the evaluated patients had new symptoms, but they were not related to PPS. Evaluation by a specialized team is recommended to avoid false positive diagnosis.

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P143 THE CLINICAL FEATURES OF AMYOTROPHIC LATERAL SCLEROSIS IN SOUTHWEST CHINA

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Keywords: clinical features, age of onset, registry

Objective: To profile clinical features of amyotrophic lateral sclerosis (ALS), we performed a large sample, cross-sectional study based on a hospital registry of ALS in Southwest China.

Methods: Patients were coded in our tertiary referral center from May 2006 to September 2014. The demographic data and disease-related parameters were collected.

Results: A total of 1131 patients were included in the study. The mean age of onset was 54.3 11.6 years and the highest proportion of age of onset (30.6%) was between 51 to 60 years old. The male to female ratio was 1.45:1. Nearly 30% of the patients were young-onset, and 20.3% of the patients were bulbar onset; only 35% received riluzole treatment. The young-onset patients had a higher educational level, with a higher proportion performing manual labor and living in rural areas, and a lower proportion with bulbar-onset compared with those who were older at onset. The bulbar-onset patients were older at the age of onset with a lower proportion of males than the spinal-onset patients. The male patients had a higher educational level, higher prevalence of history of smoking and drinking, lower proportion of bulbar-onset diagnoses, and higher ALSFRS-R total scores than did female patients.

Conclusions: Chinese ALS patients may be younger at the age of onset than Caucasian patients. Environmental and geographical factors are related to the occurrence of ALS. The large treatment gap indicated the pressing needs for medical and financial support for Chinese ALS patients.

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P144 SURVIVAL AND RISK STRATIFICATION OF ALS PATIENTS – A POPULATION BASED STUDY

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Keywords: prognosis, survival, mortality

Background: Amyotrophic Lateral Sclerosis natural history and patient risk stratification, are areas of research and development. We aimed (i) to describe the survival of a representative cohort of French ALS patients, and (ii) to identify covariates associated with various pattern of survival using a risk classification analysis.

Methods: ALS patients recruited in the FRALim register (2000-2013) were included. Time-to-death analyses were performed using Kaplan-Meier method and Cox model. A RECursive Partitioning and AMalgamation (RECPAM) algorithm analysis identified sub-groups of patients with different patterns of survival.

Results: Median survival time was of 26.2 and 15.6 months, since time of onset and time of diagnosis, respectively. Four groups of patients depending on their baseline characteristics and survival, were identified: (i) possible and probable laboratory-supported cases with a diagnosis delay >16.46 months (median survival time (MST) 46.4 months); (ii) possible and probable laboratory supported cases with a diagnosis delay ≤16.45 months (MST: 18.3 months); (iii) definite or probable cases with an ALSFRSR >36/48 (MST: 18.1 months) and (iv) definite or probable cases with an ALSFRSR ≤36/48 (MST: 8.5 months).

Conclusions: Median survival time is among the shortest ever reported among worldwide population-based studies. This is probably related to the age structure of the patients (the oldest identified to date), driven by the underlying population (30% of subjects older than 60 years). Further research in the field of risk stratification could help physician to better anticipate prognosis of ALS patients and improved methodologies for better design of randomized controlled trials.

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P145 EXPLORING RISK FACTORS FOR ALS USING THE U.S. MEDICARE DATABASE

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Background: ALS is a very rare disease, so that most observational studies of risk factors have been small with limited power. Recent pooled studies, while larger, have generally been limited to risk factors ascertained from questionnaires. The U.S. Medicare database with about 15% of the U.S. population (44 million participants, 65 years and older) provides the opportunity to explore associations between medical conditions as well as prescribed medications and ALS risk with thousands of ALS subjects. Among medical conditions, cancer has been of special interest because several studies suggest that a previous cancer diagnosis lowers risk of subsequent neurodegenerative diseases. The adoption of Medicare Part D coverage of prescription drugs effective in 2006 allows examination of associations between medications prescribed for other conditions and subsequent ALS diagnosis.

Objectives: To examine the associations between cancer overall and at various sites and subsequent ALS risk. To assess the associations between select medications, including for example, statins, and ALS risk.

Methods: In our study of cancer, we used all first primary cancer cases from the Surveillance, Epidemiology and End Results (SEER) Program (diagnosed 1992-2005), linked to Medicare claims data. Cases were followed from cancer diagnosis until the earliest date of ALS diagnosis, a break in Medicare claims data, death, age 85 or December 31, 2005. We selected a comparison group from a 5% random Medicare sample in the SEER areas who were cancer-free and censored as above, or until a cancer diagnosis. We used Cox proportional hazards models to estimate ALS hazard ratios (HRs), using age as the time scale, and adjusting for sex, race, medical surveillance, and other factors. In our study of medications, we are using a case-control design with ALS cases (diagnosed 2006-2012) frequency-matched to controls based on age, sex, race, calendar year, zip code, and available Part D (prescription drug) coverage. Odds ratios (ORs) for ALS risk after medication use are estimated from unconditional logistic regression models adjusted for the matching factors and healthcare utilization.

Results: In our cancer study, a total of 303 ALS cases were ascertained among cancer patients (2,154,062 person-years) compared to 246 ALS cases (2,467,634 person-years) in the reference population. There was no overall association between cancer and ALS risk (adjusted HR = 0.99; 95% CI = 0.81-1.22), nor by gender or race. The relationship between site-specific cancers and ALS risk was null after correcting for multiple comparisons.

Discussion and conclusions: Having had a cancer diagnosis was not associated with the risk of incident ALS. Evaluating associations between prescription drugs and subsequent ALS diagnosis in the Medicare database

may provide clues to mechanisms of motor neuron degeneration, as well as identify medications that are associated with ALS risk.

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P146 DISTRIBUTION AND PERSISTENCE OF THE CYANOBACTERIAL NEUROTOXIN BMAA: FACILITATING CHARACTERISTICS FOR HUMAN EXPOSURE

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Keywords: BMAA, cyanobacteria, persistence

Background: Aquatic, terrestrial, airborne and symbiotic cyanobacteria produce a wide range of bioactive metabolites, including potent toxins. These toxins present risks to human health via a wide range of exposure media (water, food, air) and multiple exposure routes (oral, inhalation, dermal, haemodialysis). Cases of human illness and mortality exist after exposure to several of these toxins (1). β-N-methylamino-L-alanine (BMAA) of cyanobacteria is of widening toxicological interest, not least because of the possibility that this neurotoxin is causally-associated with ALS (2).

Objectives: (i) to analyse dried herbarium specimens and fresh samples of cyanobacteria from diverse sources for BMAA and the kinetics of BMAA persistence in water; (ii) to contribute to understanding of the geographical occurrence of BMAA and the potential for long-term human exposure.

Methods: Dried herbarium specimens of cyanobacteria collected between 1837 and 1950 were analysed in comparison with freshly collected cyanobacteria from UK lakes. Samples were freeze-dried and BMAA-extracted, before analysis by HPLC with fluorescence detection and verification by triple quadrupole LC-MS/MS. Further samples of BMAA were prepared to look at the persistence of this cyanobacterial toxin in nature.

Results: Freshly collected cyanobacterial material and herbarium specimens of cyanobacteria were found to contain BMAA. The half-life of BMAA standards under different conditions ranged from about 5 to 90 days.

Discussion and conclusions: Recognition of the widespread and long-term occurrence of BMAA in diverse environments is supported by our findings of BMAA in dried herbarium samples of cyanobacteria, collected between 1837 and 1950, including from high-usage waterbodies and terrestrial sites in Chile, Germany, Sierra Leone, Tanzania, the UK and the USA. Freshly-collected water samples containing cyanobacteria and BMAA reveal the toxin to be associated with both soluble and particulate cyanobacterial fractions, with consequent implications for the effective removal of BMAA in drinking water- and haemodialysis water treatments. The persistence of BMAA in water was influenced by temperature and light, with biodegradation being evidenced. These findings are discussed with reference to the plausibility and potential for short- and long-term human exposure to BMAA of cyanobacterial origin.

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P147 A CASE-CONTROL GEOSPATIAL ANALYSIS OF RESIDENTIAL EXPOSURE TO ENVIRONMENTAL RISK FACTORS FOR ALS IN NEW HAMPSHIRE AND VERMONT – CYANOBACTERIA, AGRICULTURAL CHEMICALS, LANDFILLS AND SUPERFUND SITES

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Keywords: cyanobacteria, case-control study, risk-factor analysis

Background: There is evidence that exposures to cyanobacteria and environmental toxicants are risk factors for ALS9(1). We have reported statistically significant clusters of ALS patients in northern New England, and that living in close proximity to a lake with a high content of cyanobacteria is a significant risk factor for ALS(2-5).

Objectives: To study exposure to cyanobacteria and to sources of environmental toxicants (Superfund sites, landfills, agricultural chemicals, etc.) as risk factors for ALS in our Northern New England (NNE) ALS Repository.

Methods: Our NNE ALS Repository currently contains residential data on > 500 ALS patients from NH and VT collected over the period from 1999 to the present. We currently have lifetime residential histories from >228 neurology clinic control patients. Measures of cyanobacteria from databases of *in situ* sampling of cyanobacteria were matched with contemporaneous data from satellite remote sensing (SRS) scans of the same water body to calibrate the SRS scans. Using our complementing multiscale SRS approach we achieved R2 of 0.6-0.9 and >80% accuracy. SRS scans for all lakes > 8 hectares in NH and VT were used to estimate exposure to cyanobacteria for temporal windows prior to the diagnosis of ALS. Sources of environmental toxicants in NH and VT were collected from publicly available databases, and expanded to the level of selected chemicals. GPS coordinates of addresses of the ALS patients and controls were compared, using GIS kernel density estimation methodology, diffusion and wind-effect modeling to analyze residential proximity to these sources as potential risk factors for ALS.

Results: We have previously published a statistically significant relationship between residential location and water bodies with high cyanobacteria concentrations in NH and VT. The new case-control studies determine the odds ratios of developing ALS in relation to proximity of the place of residence to lakes with quantified cyanobacterial content and to sources of other environmental toxicants.

Discussion and conclusions: These studies explore exposure to cyanobacteria and other environmental chemicals as risk factors for ALS.

Acknowledgements: Funding sources: ALS Association grants SC5181 and 15-IIP-213. CDC contract 200-2014-59046.

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P148 A GEOSPATIAL ANALYSIS OF THE DISTRIBUTION OF ALS CASES IN FLORIDA AND ENVIRONMENTAL RISK FACTORS - CYANOBACTERIA, AGRICULTURAL CHEMICALS, LANDFILLS, AND SUPERFUND SITES

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Keywords: clusters, cyanobacteria, BMAA

Background: There is evidence that exposures to cyanobacteria and environmental toxicants are risk factors for ALS. We have reported that living within 0.5 miles of a lake with frequent cyanobacteria blooms is a significant risk factor for ALS (1-4).

Objectives: To investigate residential proximity to water bodies with cyanobacteria blooms and to sources of environmental toxicants (Superfund sites, landfills and agricultural chemicals) as risk factors for ALS.

Methods: The Florida ALS Surveillance Project of the National ALS Registry collected data on 1,451 prevalent ALS patients in the 3-year period 2009-2011 (5). We mapped residences using a geocomputation method that disaggregates the zip-code level data based on the populations distribution, calculates the age- and gender-specific count of ALS cases using the kernel density estimation, and estimates statistical significance of the difference between the count of ALS cases and the expected count, based on the data for the whole state of Florida, using a Monte Carlo process.

Measures of water quality relating to cyanobacteria were obtained from the Florida water management districts. Sources of environmental toxicants in Florida were collected from publically available databases. These sources of environmental toxins/toxicants were geomapped. Geospatial methods, including kernel density estimation, diffusion and wind-effect modeling were used to analyze residential proximity and potential exposure to these toxicants as possible risk factors for ALS.

Results: Using a 10 km bandwidth, we found five geographic areas of Florida with a statistically significantly increased frequency of ALS ($p < 0.01$), and an additional 5 areas with a statistically significantly increased frequency of ALS at the $p < 0.05$ level. A preliminary comparison of the 3-year prevalence of ALS in zip codes bordering the Indian River Lagoon (IRL), which has frequent massive cyanobacteria blooms, with that in zip codes further from

the IRL, showed rates of 9.0/105 population, and 6.6/105 population, respectively; odds ratio 1.36 (p = 0.06, not significant). We are expecting to receive patient aliased addresses for the whole of the Florida ALS database shortly, and will present additional geomapping analyses at the meeting.

Discussion and conclusions: We will report which environmental toxins/toxicants are statistically significant risk factors for ALS in Florida, and the distance over which this risk extends.

Acknowledgements: Funding sources: ALS Association grants SC5181 and 15-IIP-213. CDC contract 200-2014-59046.

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P149 ENVIRONMENTAL EXPOSURES AS RISK FACTORS FOR ALS IN THE LAKE ERIE REGION OF NORTHERN OHIO – THE CLEVELAND CLINIC DATABASE AND CYANOBACTERIA

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Keywords: clusters, cyanobacteria, Lake Erie

Background: There is evidence that exposures to cyanobacteria is a risk factor for developing ALS. We have reported statistically significant clusters of ALS patients in northern New England, and that living near lakes with cyanobacteria blooms is a significant risk factor for ALS (1-4).

Objectives: To investigate: 1) the frequency of ALS adjusted for age and gender in northern Ohio; 2) a possible relationship between observed clusters of ALS patients residences and proximity to lakes with frequent algal blooms; 3) a possible relationship between estimated cyanobacteria concentrations in northern Ohio lakes and patient addresses in the Cleveland Clinic ALS database (CCALS).

Methods: We have applied our previously reported(2-4) statistical techniques to investigate the distribution of patient residences in the CCALS database. Using databases of directly measured cyanobacteria concentrations and satellite remote sensing (SRS) spectral observations, we derive estimated cyanobacteria content of all lakes > 8 hectares in northern Ohio. Using Geographical Information System (GIS) methodology we map the addresses of ALS patients and their relationship to nearby lakes. Using kernel density estimation, diffusion and wind-effect modeling we analyze the residential exposure to cyanobacteria as a potential risk factor for ALS.

Results: Massive cyanobacteria blooms in Lake Erie have forced the closure of water facilities drawing from the lake twice in the last two years. In our CCALS database of 1,000 cases, 700+ of whom reside in Ohio, we have preliminarily found 16 areas of statistically significantly increased age- and gender-adjusted rates of ALS in northern Ohio ($p < 0.01$), and another 13 with $p < 0.05$. SRS shows massive recurrent cyanobacteria blooms adjacent to one large area of increased incidence of ALS towards the western end of Lake Erie.

Discussion and conclusions: Our preliminary studies were based on ALS patient addresses at the census ZCTA level. We are extending these studies to aliased GPS coordinates of addresses using a computer-based technique that protects confidential patient information (5), and to apply GIS mapping analysis of the risk of developing ALS at various distances from lakes and at various concentrations of cyanobacteria. Our preliminary studies support the theory that exposure to cyanobacteria is a risk factor for developing ALS.

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P150 AEROSOLIZATION OF CYANOBACTERIA AND CYANOTOXINS AS RISK FACTORS FOR ALS/ NEURODEGENERATION

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Keywords: cyanobacteria, aerosol, BMAA

Background: The primary etiologic factors leading to the sporadic (non-familial) form of ALS remain unknown. One important environmental risk factor/trigger for ALS is the cyanobacterial derived toxin, β-N-methylamino-L-alanine (BMAA), that has been previously linked to the high rates of ALS in Guam 50+ years ago.

Objectives: To examine the aerosol route of ALS patient and control exposure to cyanobacteria. We hypothesize that tissue samples from subjects with such exposures will have cyanobacteria in the respiratory tract that are detectable by PCR and ELISA assay and that postmortem tissue from available subjects will have detectable levels of BMAA in CSF, brain and spinal cord.

Methods: We are determining exposure by 1) collecting on filters aerosol from lakes with cyanobacteria blooms and analyzing the filters for cyanobacteria using fluorescence microscopy, DNA analysis using PCR, and an ELISA assay for cyanotoxin microcystin (MC); 2) evaluating nasal swabs and bronchoscopy samples on selected high risk exposure subjects (ie those living close to lakes with cyanobacterial blooms) vs. low risk subjects controls (ie those living far from lakes with cyanobacterial blooms) using fluorescence microscopy, DNA analysis and MC levels. MC levels are being measured in blood with ELISA testing. All who undergo bronchoscopy are asked to fill out our specially designed questionnaire that records risk factors and demographic information; 3) evaluating postmortem lung with fluorescence microscopy and PCR for detection of cyanobacteria and brain, spinal cord and cerebrospinal fluid with analytical techniques (triple quadrupole mass spectrometer with an Ultra High Pressure Liquid Chromatography) for BMAA.

Results: Preliminary results show evidence of cyanobacteria in lung tissue of high risk individuals (both controls and ALS patients) by fluorescence microscopy and DNA analysis. Aerosol filters show the presence of cyanobacteria by fluorescence microscopy, DNA testing and MC analysis. We are developing algorithms to model data derived from aerosol filters to impute likely human exposures, and to relate these to findings in nasal swabs, bronchoscopy samples and autopsy specimens of lung tissues.

Conclusion: Our preliminary studies indicate that aerosolization of cyanobacteria and cyanotoxins is a potential risk factor for ALS. We are investigating the hypothesis that the risk of developing ALS increases with dose and duration of exposure to BMAA in genetically susceptible individuals through aerosolization from cyananobacterial blooms.

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P151 BAYESIAN MODELLING OF POTENTIAL ASSOCIATION BETWEEN SOIL MINERAL LEVELS AND SMALL AREA SPATIAL RISK OF ALS IN IRELAND

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Keywords: incidence, soil minerals, spatial analysis

Background: We have recently mapped ALS spatial risk in Ireland using Bayesian and cluster analysis methods and electoral division (ED) and small area (SA) levels. Here we extend this analysis to include soil mineral levels from the Irish National Soils Database as Bayesian conditional auto-regression covariates to determine associations with ALS SA risk.

Methods: Data on 45 different soil parameters were obtained under license from National Soils Database (via Irish EPA). We interpolated average values of each soil constituent for each small area using ordinary Kriging. All cases of ALS in Ireland from January 1995 to December 2013 were identified from the Irish ALS register and observed and age & gender standardised expected cases were calculated for each SA.

Besag-York-Mollié (BYM) models were then built including each parameter from the national soils database in turn as a Bayesian covariate in the BYM model. Models were compared using the deviance information criterion (DIC) and separate models were built for ALS subtypes.

Results: 1,701 ALS patients were included - 959 (56%) were male, 938 (55%) had limb onset ALS. 315 Bayesian models were built in total. Of the 315 models built, only one resulted in a coefficient that did not overlap zero. For limb onset cases, total magnesium had a mean coefficient of 0.319 (credible interval 0.033 - 0.607).

Discussion: We report the first spatial analysis of potential association between ALS and soil minerals using a population-based dataset collected over 18 years. Our sole non-zero finding is likely a random finding due to the high number of models built. This is congruent with findings in Guam, which showed that despite high levels of soil aluminium and low levels of calcium in the soil, there was no difference in exposure via food when compared to other locations with lower rates of ALS.

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P152 OCCUPATIONAL EXPOSURE TO LEAD, AGRICULTURAL CHEMICALS AND ELECTROMAGNETIC FIELDS AND FUNCTIONAL PARAMETERS AT ALS DIAGNOSIS

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Keywords: occupation, lead, chemicals

Background: The evidence linking occupational exposures to lead, agricultural chemicals and electromagnetic fields (EMFs) to ALS is inconsistent, perhaps due to differences in exposure assessment.

Objective: To evaluate the associations between lifetime occupational exposures to lead, agricultural chemicals and EMFs and function at diagnosis in ALS-COSMOS.

Methods: Data were collected at the first clinical evaluation and via telephone questionnaire. A detailed lifetime occupational history was collected using a standard questionnaire, and all occupations held for more than one year, including those from military service, were coded for exposure by an occupational hygienist. For occupational exposures to lead and agricultural chemicals, exposure was coded as no exposure, possible or probable. Exposure to EMFs were classified on a scale from 1-4 based on a semi-quantitative job exposure matrix for power frequency magnetic fields. We calculated duration of exposure as a proxy for dose, latency from the first exposure to symptom onset, and latency from the last exposure to symptom onset. Linear regression methods were used to estimate associations between each exposure measure and either baseline ALSFRS-R or % Forced Vital Capacity (%FVC), adjusting for sociodemographic covariates.

Results: 355 patients were enrolled, of whom 324 and 325 had ALS-FRS and FVC data, respectively. Mean ALS-FRS-R and %FVC (+ standard deviation (SD)) were 36.0 (+ 6.76) and 79.2 (+ 22.6)%, respectively. Average age was 61.1 (+ 10.3) years. 61% were male, 11% were non-white and 6.5% were Latino. The median duration of symptoms prior to diagnosis was 11.5 months. The number of jobs held ranged from 1 to 14, with an average of 5.9 (+ 2.7). Mean duration of work was 39.0 (+ 10.2) years (range 7.0-64.6 years). The proportion of those working in jobs with possible or probable exposure to agricultural chemicals or lead declined from 18.4% to 3.3% and from 11.4% to 3.3%, respectively, for the first job to the job closest to the time of diagnosis. The proportion working in jobs with EMF exposure was between 1.2 and 3.1%, with no clear pattern. We found no associations between any measure of occupational exposure and either functional measure.

Discussion: Our results may illustrate a healthy worker effect' as persons exposed to these agents for long periods of time have greater physical reserves' than those not exposed; due to the requirements of such occupations they may be in better physical condition and present at diagnosis with better function even after controlling for symptom duration. Alternatively, these exposures may only be associated with the onset of ALS. Finally, these exposures may not be associated with ALS function at baseline, but may be associated with the rate of ALS progression.

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P153 TEMPORAL ASSOCIATION BETWEEN A FORWARD LAG IN TOTAL HISTORICAL ACCUMULATED PETROL (GASOLINE) LEAD EMISSIONS AND THE PERCENTAGE OF MND AS A CAUSE OF ALL DEATHS IN AUSTRALIA

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Keywords: lead, bone, trend

Background: In Australia between 1979 and 2011 motor neuron disease (MND) as a percentage of total deaths increased from 0.19 to 0.46 percent. It has been suggested that genetics could not have played a causal role in the increased rate of MND deaths in such a short period of

time. If the increased rate of deaths was not caused by more awareness of MND, some other factor must be responsible. It has been suggested that an environmental factor could be the cause of the increasing rate of MND as a cause of death in Australia.

Atmospheric emissions of lead to the entire Australian continent from leaded petrol have been calculated to total 240,510 tonnes over seven decades of use. The lead emitted from automotive tailpipes was deposited into urban soils and dusts with a majority of the lead still present within the surface soil. Lead in urban soils has a half-life of approximately 700 years. This lead contaminated surface soil is resuspended into the atmosphere during dry periods and fine lead enriched soil dust migrates into homes. In addition, lead contaminated soils are tracked into homes via shoes and attached to fur on pets.

Objectives: The objective of this study was to analyse the statistical link between MND as a percentage of all causes of death in Australia, with a major environmental contaminant signal introduced into the environment - historical petrol lead emission trends.

Methods: The association between various forward lags of accumulated petrol lead emissions (between 10 and 24 years) and the percentage of MND of all causes of deaths between 1979 and 2011 were examined using linear regression.

Results: The MND percentage of all deaths and total historical accumulated petrol lead emission are associated strongly with a best fit forward lag of an accumulated petrol lead emission period of 20 years (R^2 = 0.96, p = 1.47 E-23).

Discussion and conclusions: It is hypothesised that MND develops approximately 20 years following the bioaccumulation of lead in human bones from exposure to petrol derived lead that has accumulated in urban soil dust. The literature supports the hypothesis that lead in bones is associated with motor neuron diseases such as ALS. MND percentage of all cause death and total historical accumulated petrol lead emission are associated strongly. Should further studies confirm that exposure to lead from past emissions of lead in petrol and its accumulation in soils and house dusts is causally related to the development of MND, then lead contaminated urban soils and house dusts may require extensive remediation or isolation to prevent further development of MND in future generations.

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P154 ENVIRONMENTAL RISK FACTORS FOR ALS IN NH AND VT - A QUESTIONNAIRE-BASED CASE-CONTROL STUDY

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Keywords: risk factors, BMAA, methylmercury

Background: Sporadic ALS (sALS) is thought to result from exposure to environmental risk factors in susceptible individuals.

Objectives: To investigate environmental risk factors for sALS in a questionnaire-based case-control study of the Northern New England (NNE) ALS Repository.

Methods: We examined potential ALS risk factors in a preliminary analysis of the data from a specially designed questionnaire based on the ACES modules of 157 ALS patients and 373 controls (combining clinic controls with a population-based control group (2). Analyses were adjusted for age and gender. We have launched a formal case-control study evaluating environmental risk factors using this questionnaire. The Northern New England study of Environmental Exposure, Toxins and Neurological Disease currently contains 436 ALS patients, and in addition we have collected completed questionnaires from 228 neurology clinic controls.

Results: In our preliminary analysis, the following items were statistically significant at P<0.05 level: residing near a waterbody at the time of diagnosis, odds ratio 1.97; estimated methylmercury consumption in fish in upper quartile vs. lower quartiles, odds ratio 6.08. The estimated annual mercury consumption based on self-reported fish consumption categorized by species and typical mercury levels reported by the US EPA was significantly higher in ALS patients (770 μg/yr), compared to either the neurology clinic controls (390 μg/yr), or to the general-population-controls (510 μg/yr). The following showed non-significant trends of increased odds ratios from 1.20 to 1.58: (ever having lived near algal blooms; watersport participation; and self-reported exposures to lead and to mercury from hobbies and occupations).

Discussion and conclusions: These results suggest exposure factors that may be associated with ALS. Analysis of our full cohort, as well as replication in other populations will provide a more definitive assessment of ALS risk factors.

Acknowledgements: Funding sources: ALS Association grants SC5181 and 15-IIP-213. CDC contract 200-2014-59046.

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P155 RETROSPECTIVE ANALYSIS OF HEAVY METAL TESTING IN ALS

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Keywords: heavy metals, toxicology, phenotype

Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, characterized by progressive degeneration of motor neurons. The cause of sporadic ALS is still unknown. Metal toxicity has been proposed as a possible environmental risk factor for developing ALS. Furthermore, case reports of clinical syndromes that resemble ALS following exposure to heavy metals have been described. For this reason, heavy metal blood testing is often used in the ALS clinic during the diagnostic period.

Objectives: To review current practices of heavy metal testing in the MNH ALS Clinic, to determine if there is an increased incidence of patients with elevated heavy metals in our patient population, and to examine if their ALS phenotype or progression varies as compared to the literature.

Methods: Retrospective chart review looking for heavy metal testing was conducted for 436 ALS patients seen in the MNH ALS Clinic between January 2006 and December 2014. Results for mercury, lead, arsenic, zinc, copper and selenium testing were collected. In depth chart reviews were conducted on those patients who had elevated levels. Information on symptom onset, diagnosis, phenotype and any referrals to toxicology were examined.

Results: Of the patients examined, 53% had heavy metal testing done, with 20.8% of cases having elevated results. The demographics (gender and urban/rural residence) did not differ between those with elevated heavy metal levels and those with normal levels. ALS phenotype in patients with elevated heavy metals was comparable to general ALS phenotype population in the literature. Several patients with referrals to toxicology were examined as case studies. Change in diet nor chelation therapy altered their course of disease progression.

Discussion and conclusions: A relatively high proportion of the patients in the MNH ALS Clinic have had elevated heavy metal levels, yet preliminary analysis shows no clear effect on phenotype; however, testing practices have not been consistent. We propose to establish a control group for further testing, and also to discuss the appropriateness of normal reference ranges with laboratory medicine.

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P156 POPULATION-BASED RISKS FOR CANCER ASSOCIATED WITH ALS CASES

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Keywords: cancer, neurodegenerative disorders, computerized genealogy

Objective: To estimate the risks for cancer (overall and site specific) in the ALS proband and enhance our understanding of the pathogenesis of ALS.

Methods: ALS and cancer cases were identified in a computerized Utah genealogy linked to a statewide cancer registry. Relative risks were estimated as the ratio of observed to expected number of cancers in a set of cases. Site-specific rates for cancer were estimated for sex-, birth year- (5 year birth year range), birth state- (Utah or not), and birth county- (urban or rural) cohorts and were used to estimate the expected number of cancers among ALS cases. Due to assumed short life span, Cox regression was used to include years at risk in estimation of cancer risks for ALS cases.

Results: We found an overall decreased hazard ratio (HR 0.79, P=0.017, 95% CI 0.64-0.96) for cancer of any site in 884 deceased ALS patients. We observed significantly increased hazard for salivary gland cancer (HR 5.86, P=0.032, 95% CI 1.02-17.48) and testicular cancer (HR 5.48, P=0.014, 95% CI 1.50-14.12). We additionally identified suggestive evidence for increased hazard for skin melanoma (HR 1.78, P=0.09, 95% CI 0.9-3.11) in those who died from ALS. Significantly decreased risk was observed for lung cancer (HR 0.18, pp=.003, CI 0.02, 0.63).

Conclusions: Our study uniquely provides evidence that ALS, like other neurodegenerative diseases, including PD and Alzheimer's, may be protective against cancer. We have identified salivary and testicular cancers as cancers observed in excess in ALS cases. We confirm prior findings of potentially increased risk for melanoma.

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P157 APOE GENOTYPE AND ONSET OF COGNITIVE IMPAIRMENT IN ALS: NO CORRELATION. A POPULATION-BASED STUDY

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Keywords: ApoE, FTD

Background: The relationship between the apolipoprotein E (ApoE) ε4 genotype and an increased risk of developing Alzheimer's disease (AD) is well established. In frontotemporal dementia (FTD), the second most frequent dementia disorder, an increased risk in subjects carrying the ApoE ε4 genotype has also been reported. No data are available the ApoE ε4 genotype and the risk of developing FTD in ALS patients.

Objectives: To assess the relationship between ApoE genotypes and ALS with comorbid FTD in a population-based series of Italian ALS patients.

Methods: A consecutive series of ALS patients incident in Piemonte underwent an extensive cognitive evaluation. Patients were classified according to the Strong *et al* criteria in ALS-FTD, ALS with cognitive impairment (ALS-Ci), ALS with behavioural impairment (ALS-Bi) and ALS cognitively normal (ALS-CN). ApoE genotypes were assessed with the standard methodology. A total of 179 age- and gender-matched population-based controls were also genotyped for ApoE.

Results: A total of 282 ALS patients have been included in the study: 141 (50%) were classified as ALS-CN; 85 (30.2%) as ALS-Ci; 18 (6.4%) as ALS-Bi and 38 (13.5%) as ALS-FTD. ApoE ε4 genotype was ε4 and ε2 genotypes in ALS patients were not related to their site and age at onset. The frequency of ApoE ε4 genotype in ALS cases (13.6%) was similar to that observed to population-based controls (12.2%) (p=0.41). The frequency of ApoE ε2 genotype in ALS cases (12.4%) was similar to that of controls (12.4%).

Conclusions: We did not find any correlation between ApoE genotype and the risk of developing FTD in our series of population-based ALS patients. While ApoE is an established risk for AD and pure FTD.

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Theme 9 Genetics

P158 ALS ONLINE GENETICS DATABASE, ALSOD: NEW FEATURES AND CURRENT POSITION

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Keywords: ALSoD, web bases, database

ALSoD offers unrestricted online access to a growing database of genetics data with records selected and curated from publications. It is an integrated tool set for comparing, picturing, analysing and sharing both publicly available and user-discovered data from well-known labs across the globe. As at June 2015, ALSoD has a collection of 126 genes, 657 mutations and 1093 patient records. We have presented several features to expand usability by including fresh navigation menus. We developed a page to compare ALSoD with ALSdb (a database of 1,424 Caucasian sequenced data) and to report findings graphically. Another page summarises the discovery methods of genes by the years they were found. This article provides an update to the ALS Online genetics database which is sustained by a joint approach of automated analyses, collaboration and manual curation to produce an up-to-date depiction of the genetic data and links across associated sources of information. Findings and huge data generated from linkage analysis, GWAS, Next generation sequencing are at the helm of our current understanding of neurodegenerative diseases and at the basis of contemporary drug advancement strategies thereby revealing a noteworthy impact on the ratio of patients with a genetic cause.

Acknowledgement: We are especially grateful for the long-standing and continued funding of this project from the ALS Association and the MND Association of Great Britain and Northern Ireland. We also thank ALS Canada, MNDA Iceland and the ALS Therapy Alliance for support. The research leading to these results has received funding from the European Community's Health Seventh Framework Programme FP7/2007-2013 under grant agreement number 259867. We thank the NIHR specialist Biomedical Research Centre for Mental Health and the Biomedical Research Unit for Dementia at the South London and Maudsley NHS Foundation Trust (SLaM) and the Institute of Psychiatry, King's College London. Aleks Radunovic, Nigel Leigh, and Ian Gowrie originally conceived ALSoD. ALSoD is a joint project of the World Federation of Neurology (WFN) and European Network for the Cure ALS (ENCALS).

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P159 COMPREHENSIVE GENETIC SCREENING OF 28 ALS-RELATED GENES IN A JAPANESE ALS COHORT

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Keywords: next-generation sequencing, genetic epidemiology

Background: Comprehensive genetic epidemiology in Japanese ALS patients has not been sufficiently analyzed.

Objectives: To investigate the frequency and contribution of variants in the 28 known ALS-related genes in Japanese ALS patients from a large ALS cohort, using next generation sequencing technologies.

Methods: This study included 508 (39 familial and 469 sporadic) ALS patients who had participated in research conducted by the Japanese Consortium for Amyotrophic Lateral Sclerosis (JaCALS). We designed a multiplex, PCR-based primer panel to amplify the coding regions of 28 ALS related genes; as follows, *SOD1, ALS2, SETX, SPG11, FUS, VAPB, ANG, TARDBP, FIG4, OPTN, VCP, UBQLN2, SIGMAR1, DAO, NEFH, DCTN1, TAF15, EWSR1, PRPH, GRN, CHMP2B, ZNF512B, PFN1, ATXN2, TFG, C9orf72, RNF19A* and *SQSTM1*. We sequenced DNA samples from 257 Japanese ALS patients using an Ion Torrent PGM sequencer. We also performed exome sequencing and identified variants of the 28 genes in an additional 251 ALS patients using an Illumina HiSeq 2000 platform. To determine if any sequence variations were novel or known variants, all non-synonymous variants were screened with dbSNP, the Human Gene Mutation Database (HGMD), and the Human Genetic Variation Database (HGVD). We picked up known ALS pathogenic variants, and then, the functional consequences of the novel and non-synonymous variants were predicted in silico using the SIFT and PolyPhen2 software. These variants were confirmed by Sanger sequencing.

Results: Known pathogenic variants were identified in 19 (48.7%) of the 39 familial ALS patients and 14 (3.0%) of the 469 sporadic ALS patients. Six familial ALS patients (15.4%) and 32 sporadic ALS patients (6.8%) harbored one or two novel non-synonymous variants of ALS-related genes that might be deleterious.

Discussion and conclusions: We have performed the first comprehensive genetic screening of ALS-related genes in a large Japanese ALS cohort. We found that 6.5% of Japanese ALS patients (48.7% of familial and 3.0% of sporadic ALS patients) harbor known pathogenic variants of ALS-related genes. 32 patients (6.8%) carried one or more novel and potentially pathogenic variants of an ALS-related gene. These results suggest that variants of known ALS-related genes might play important roles in sporadic ALS, and would be useful for strategies of genetic screening and counseling in these patients.

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P160 GENETIC ANALYSIS OF CBL-C GENE AS A CANDIDATE GENE FOR SPORADIC AMYOTROPHIC LATERAL SCLEROSIS IN AFRICAN AMERICANS

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Keywords: Cbl-c, Ubiquitin Proteasome System (UPS)

Sporadic amyotrophic lateral sclerosis (SALS) accounts for 90% of all cases of ALS. Aetiology of SALS is largely unknown and it is not well studied in African Americans. SALS is hypothesized to be a complex disorder in which disease is modulated by variations in multiple genetic loci interacting with each other and perhaps environmental factors. We have shown that the ubiquitin proteasome system (UPS) may be directly involved in the pathology of ALS. Cbl proteins negatively regulate receptor and nonreceptor tyrosine kinases by functioning as ubiquitin protein ligases that mediate the ubiquitination of activated tyrosine kinases. Therefore, mutations in *cbl-c* gene may affect degradation of tyrosine kinases in motor neurons, thus leading to motor neuron diseases.

In this study we investigated association of SALS in African-Americans to a coding region cytosine duplication (dupC) mutation in *CBLC*. This variation has very low frequency in people of European descent, but common in Africans. It leads to frame-shift and premature stop codon. We analyzed dupC variant in a case-control cohort of 356 African-Americans for association with SALS. DNA samples extracted from whole blood from our own collection and others obtained from Coriell Cell Repositories were screened using four primers PCR reaction for the presence of dupC mutation in *cbl-c*. We investigated the association between sporadic ALS and the mutation using X^2 analyses. Forty percent of African American samples were found to contain the dupC mutation. However, we found no significant difference between polymorphism frequency in the disease samples as compared to controls (p=0.34).

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P161 MUTATION SCREENING IN BRAZILIAN ALS PATIENTS - AN ANALYSIS OF 7 GENES IN FAMILIAL AND SPORADIC CASES

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Keywords: Genetic Screening, VAPB, Familial ALS

Background: Amyotrophic Lateral Sclerosis is the most common form of motor neuron disease. Around 10% of cases are classified as familial (FALS), with more than one

affected family member, while the remaining 90% are classified as sporadic (SALS).

Method: In order to better characterize the genetic causes of ALS in patients of Southeast Brazil, 7 genes - *SOD1, FUS, TARDBP, SETX, SPG11, FIG4 and VAPB/C* – were screened using a next-generation sequencing panel from the MiSeq (Illumina) platform. A cohort of 67 patients (49 familial, 11 sporadic and 7 of unknown family history) and 11 unaffected family members were analyzed.

Results: Of these, 31 (46%) presented the c.166 C>T (p.P56S) mutation in the *VAPB/C* gene, being thus diagnosed with ALS8 (1); 4 patients (6%) carried mutations in *SOD1* (c.G50C; c.T116G; and c.T380C in two patients), with one of them also carrying a seemingly damaging mutation in *FIG4* (c.G2440T); 1 patient presented a previously known *FUS* mutation (c.C1558T) (1.5%); and 1 patient presented two mutations in trans in the *SPG11* gene (c.6477+4A>G and c.6365_6387del) (1.5%). Mutations of unknown clinical significance in *SETX* and *SPG11* were also found. None of the sporadic patients had a causal mutation discovered by the present panel.

Considering solely the familial individuals, we were able to diagnose 63% of the ALS cases. None of the unaffected members showed any of the evaluated mutations. The main reason for the larger proportion of familial cases in this cohort is due to patient referral to our center for genetic investigation. The high rate of P56S mutation in the *VAPB/C* gene shows the spreading of this mutation in Brazil and highlights the importance of our findings describing this mutation 11 years ago(1). Additionally, previous haplotype studies with distinct ALS8 families suggested that P56S has a founder effect and that patients are clustered in Southeast Brazil (2).

Discussion and conclusion: The present work shows that screening of 7 genes responsible for FALS allowed to confirm the diagnosis in 33% (6/18) of the non-ALS8 familial patients. The unique genetic diversity found in Brazil features the importance of studies investigating ALS genetics in this population.

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P162 NEK1 MISSENSE VARIANTS IN 252 GERMAN AND NORDIC ALS FAMILIES

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Keywords: NEK1, Exome sequencing, familial ALS

Background: Most recently an association between heterozygous LoF variants of *NEK1* (NIMA-related kinase 1) and ALS has been suggested by whole exome sequencing (WES) of mostly sporadic ALS (sALS) patients (1). NIMA-related kinases (NEKs), such as NEK1, represent an evolutionarily conserved family of serine/threonine kinases which have functions in cell cycle control, cilia regulation, DNA double-strand repair and neuronal development. Through proteomic search for NEK1-interacting proteins an association was found between *NEK1* and the ALS-proteins ALS2 and VAPB. In men, homozygosis or compound heterozygosis for deleterious NEK1 mutations causes lethal short-rib thoracic dysplasia syndromes hallmarked by premature death before or at birth. Analogously, mice with homozygous deletion of *NEK1* show polycystic kidney disease, craniofacial anomalies, and growth reduction.

Methods: Curious about the occurrence of *NEK1* mutations in familiar ALS patients we performed whole exome sequencing of 252 familial ALS and 827 control individuals. We used a gene-based test (CMC test) to analyze missense variants at two allele frequency thresholds (minor allele frequency (MAF) between 1% and 5%, and MAF less than 1%). LoF variants, defined as nonsense, canonical splice site, read-through and frameshift variants, were investigated separately (MAF less than 5%).

Results: Gene-based rare variant analysis identified an enrichment of rare genetic missense variants of *NEK1* (MAF<0.01) at single-gene level in fALS patients (dominant model, p-value = 0.0047; not significant at a genome-wide level). Compared to the previous studies we did not find an association for LoF variants in *NEK1*. Similar to the previous study we observed a relatively high prevalence of missense variants in our controls and in public databases hinting at a small penetrance of *NEK1* mutations. In addition, analysis of pedigrees of the affected families showed non-segregation with disease phenotype with regard to several of the rare variants. The rare missense variants found in this study were localized both in the regulatory and the kinase domain of the protein. Average onset of disease of patients bearing *NEK1* variants was 52 years, patients suffered from bulbar or spinal phenotype. One patient carried a deleterious mutation in the *CHCHD10* gene in addition to a *NEK1* missense variant suggesting an oligogenetic basis in some fALS patients. The other family members of this pedigree are currently being tested for the respective *NEK1* variant.

Conclusion: In summary, this is the first study examining *NEK1* variants in a large cohort of familial ALS patients. We found an association between rare missense variants of *NEK1* and fALS. Due to reduced penetrance and missing co-segregation, we hypothesize that mutant *NEK1* rather

is a risk factor for ALS than a monogenic ALS gene. Future studies must further confirm the association between *NEK1* and ALS.

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P163 FRAGMENT ANALYSIS IDENTIFIES ATYPICAL C9ORF72 (G4C2) EXPANSION PATTERNS IN FAMILIAL AND SPORADIC ALS AND ALS/FTD IN A NORTH AMERICAN POPULATION

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Keywords: C9ORF72, repeats, diagnosis

Background: A hexanucleotide (G_4C_2) repeat expansion mutation on Chromosome 9 Open Reading Frame 72 (C9ORF72; C9) has been currently identified as the most common cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). We have been actively genotyping one of the largest North American ALS and ALS/FTD cohorts since originally establishing genetic linkage at chromosome 9p21.3–9p21.

Objectives: The overarching goal of our studies is to gain a more thorough understanding of the pathogenic mechanisms associated with the C9 (G_4C_2) repeat expansion mutation, with the hope of identifying disease-causing pathways that may one day be exploited for therapeutic intervention.

Methods: To date, we have screened more than 500 FALS and 1400 SALS cases with and without FTD, not previously found to carry other known ALS-causing mutations, for the C9 expansion mutation using the repeat primed-polymerase chain reaction (RP-PCR) method.

Results: Of the samples analyzed, 50% FALS and 5% SALS cases appear to be C9 expansion carriers evident by >23 repeats, values consistent with previously published reports. Typically, electropherograms from expanded C9 carriers are described as displaying a characteristic saw-tooth pattern with 21 peaks, where each successive peak differs in one (G_4C_2) unit that decreases in signal intensity (SI) as fragment size increases. Interestingly, we identified that 40% of our expanded C9 cases do not generate electropherograms displaying a typical saw-tooth pattern. Instead, we detect an unreported atypical pattern that is characterized by a series (<21) of equally robust peaks, followed by several additional peaks (>21) of low SI that requires manipulation of the SI range for adequate detection. We have confirmed that these samples are indeed expanded using a non-radioactive Southern blot method that utilizes a digoxigennin (DIG)-labeled C9 probe.

Discussion and conclusions: What causes a difference in expansion pattern is currently unknown and is being investigated by our lab. It is possible that atypical expansion carriers have interruptions present in the (G_4C_2) tract as seen in other expansion disorders. Additionally, several other labs have reported the presence of mutations in the low complexity sequence region following the GC-rich tract. Mutations with in or following the (G_4C_2) tract may prevent efficient primer annealing and/or elongation of the template during the PCR reaction resulting in premature reaction termination. It is interesting to speculate whether variances in the expansion pattern correlate with differences in disease phenotype.

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P164 C9ORF72 REPEAT EXPANSION DETECTION USING SHORT-READ WHOLE-GENOME SEQUENCING DATA

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Keywords: C9ORF72, repeat expansion, NGS sequencing

Background: The hexanucleotide repeat expansion of CCGGGG in the *C9ORF72* gene has been recognized as a major cause of ALS and Frontotemporal Dementia (FTD) and been implicated in other neurodegenerative and psychiatric disorders. While the exact pathological cut-off has not yet been established, the *C9orf72* repeat is considered expanded when it counts more than 30 hexamers (>180bp). Since it is so prevalent, all genetic studies in ALS and FTD must be stratified or adjusted for *C9orf72* repeat status. Moreover, the exact length of the repeat expansion is difficult, if not impossible, to determine and currently involves a combination of RP-PCR, Southern blotting and targeted sequencing.

Objectives: To accurately determine the length of any 3-6 nucleotide repeat, even when they are extremely expanded, in paired-end whole-genome sequence data.

Methods: Sequencing was performed on 349 individuals (including 77 with the C9orf72 expansion) on an Illumina HiSeq 2000 using 2x100bp paired reads to ~40x depth. The tool first extracts all reads that belong to the repeat of interest by searching for 1) reads that span the repeat and contain flanking sequence on both sides, 2) reads with flanking sequence on one side, 3) read pairs of which, one

read consists entirely of the repeat, and 4) read pairs of which both reads consist entirely of the repeat. The number of repeat units are counted and normalized using the average genomic read depth to calculate the length of the repeat.

Results: Of the 77 *C9orf72* samples that RP-PCR identified as expanded, only 1 sample had a repeat count of only 2 according to the tool, whereas 6 samples had a repeat count between 20 and 30 and the rest of the samples had a repeat count higher than 30. Of the 272 *C9orf72* samples that were not expanded, only 1 sample had a repeat count of 260 according to the tool, whereas 2 samples had a repeat count between 20-30 and the rest of the samples had a repeat count lower than 20. If a count of 30 is the pathological cutoff, sensitivity of the tool is 90.9%, and specificity 99.6%. If the true cutoff is 20, these numbers are 98,7% and 98.9% respectively.

Discussion and conclusions: Further validation of this tool is ongoing in independent samples and for other repeat expansions, which will be presented. Since microsatellite expansions are a common genetic risk factor in neurodegenerative diseases, accurately determining the length of a repeat at known genomic locations using NGS data and also discovering unknown repeat expansions will allow studying the impact of repeat expansion on neurodegenerative disorders.

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P165 UPDATE ON C9ORF72 REPEAT EXPANSION IN ITALIAN ALS PATIENTS

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Keywords: C9ORF72, genetics, clinics

Background: ALS is a complex disease. Approximately 10-15% of cases are familial (FALS), while the majority are sporadic (SALS) (1). Twenty genes have been now reported to be disease causative and the *C9ORF72* hexanucleotide repeat expansion has been found in patients with familial FTLD (3-48%) and ALS (3-46%), with sporadic FTLD (2-23%) and ALS (0.4-21%), and with the combined syndromes (10-88%) (2).

Phenotypes associated to expansion are extremely variable and the variant is considered fully penetrant around 80 years (3); such clinical differences may be due to variability instability in expansion length and environmental/genetic modifiers (4).

Objectives: Determination of *C9ORF72* expansion frequency in our cohort of 840 Italian ALS patients and clinical report of mutated cases.

Methods: *C9ORF72* expansion was analyzed through repeat-primed PCR (5) performed in duplicate for all patients. Fragments length analysis was carried out on an ABI 3730 Genetic Analyzer and data were analyzed through the GeneMapper software (Version 4, ABI).

Results: Two groups of ALS expansion carriers were identified in our cohort. Group 1: repeats number > 30; group 2: repeats number of 25/30 and the typical sawtooth pattern. Expansions explain 30.2% of FALS and 4.1% of SALS cases, thus totally accounting for 5.5% of ALS.

Group 1 consisted of 10 FALS patients (3 males; 7 females) with a mean age at onset of 53.4 years and FTLD reported for only 1 female subject, and 29 SALS individuals (15 males; 14 females; mean age at onset of 56.1 years; FTD in 1 male and 1 female). Moreover, 23% of patients presented a positive family history for isolated FTLD and 7.7% for Alzheimer disease. Group 2 consisted of 3 FALS (2 males; 1 female) with a mean age at onset of 53.1 years and FTLD in 1 male subject, and 4 SALS (2 males; 2 females) with a mean age at onset of 64.2 years and no cases with FTLD.

Discussion and conclusions: ALS associated to FTLD was recorded in 8.7% of ALS expansion carriers; furthermore a positive family history for FTLD and Alzheimer's was reported for 26.1% of cases, while pure ALS without any familiarity was present in 67.4% of carriers.

C9ORF72 expansions represent the commonest mutation in our population of Italian ancestry; however, the role of other modifier genes cannot be excluded in the modulation of disease expression.

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P166 C9ORF72 EPIGENETIC MODIFICATIONS IN ITALIAN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Keywords: C9orf72, methylation, gene expression

Background: The hexanucleotide repeat expansions (RE) in the non-coding region of *C9orf72* are by far the most frequent cause of both familial and sporadic ALS and FTD. The basis for *C9orf72* carriers clinical heterogeneity remains unknown. *C9orf72* promoter methylation and histones trimethylation have been associated with transcriptional silencing and with decreased formation of RNA foci and dipeptide repeat protein aggregates. These findings suggest that these epigenetic changes have a neuroprotective role, reducing cells vulnerability to stressors and resulting in a less severe disease. Recently, also the methylation of the RE has also been reported, but more studies are needed to understand the role of such a modification in this scenario.

Methods: To consolidate the correlation between *C9orf72* methylation and its gene expression and to evaluate its role as disease modifier, we studied *C9orf72* promoter methylation in our cohort of 48 C9ALS patients using bisulfite conversion followed by sequencing.

Results: We observed methylation in the analysed region in 65% of C9ALS patients, compared to only 9% of other ALS individuals. After detecting the average methylation of samples, we estimated with extreme accuracy the frequency of methylation at each CpG site in highly methylated samples using, for the first time, next generation sequencing. In doing so we extended our analysis to 7 additional CpG sites located at 5' of CpG island, in the CpG shore. As a result, within each sample the frequency of methylated alleles was variable among different CpG sites and very high methylation was more frequent at the beginning of the target region, especially in the new CpG sites that we included in the analysis. The methylation never exceeded 50% in any of the samples, suggesting that it involved mainly only one of the two alleles. This was later confirmed in patients heterozygous for rs200034037, in which we were able to distinguish the two alleles. By performing Q-PCR on blood RNA, we found a positive correlation between *C9orf72* promoter methylation and lower levels of total *C9orf72* mRNA compared to both unmethylated C9ALS and C9-negative patients, with a different expression profile of the three *C9orf72* isoforms. While V1 and V2 expression diminished in unmethylated C9ALS and was further down-regulated in methylated C9ALS individuals, V3 expression was significant lower only in methylated C9ALS compared to C9-negative ALS patients. Methylation levels did not correlate with age at onset, but they were mildly correlated with disease duration and inversely associated with RE length, determined by Southern blot.

Conclusions: Our data supports the current hypothesis that epigenetic silencing of mutant *C9orf72* is associated to diminished levels of expanded mRNAs prone to generate pathologic effects. Since *C9orf72* methylation was associated with decreased DPR aggregates formation, we also plan to evaluate DPR levels in CSF of C9ALS patients.

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P167 CLINICAL AND GENETIC ANALYSIS OR GENOTYPE-PHENOTYPE CORRELATIONS WITH FAMILIAL ALS OF CHINA

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Keywords: gene mutation, clinica features, genotype-phenotype

Background and objectives: Our objective was to determine the distribution of the most commonly mutated genes (SOD1, C9orf72, TARDBP and FUS) and genotype/phenotype associations in Chinese FALS patients.

Methods: We collected 103 ALS families from 2003 to 2012 in Peking University Third Hospital. 58 families have been screened originally for SOD1 molecular genetic analysis, 39 SOD1-negative probands were screened for TARDBP, FUS and C9ORF72 repeat expansion in all samples using a repeat-primed PCR assay and sequence. Ultimately, we performed phenotype-genotype correlations with mutation in Chinese patients with FALS.

Results: 103 families came from 28 provinces, with bulbar-onset accounting for 10.5%, and the rest limb-onset. 76 families included 24 affected individuals (73.8%), while 27 had > 5 (26.2%), particularly in 10 pedigrees had > 10 in 46 generations (9.7%). The total FALS patients from 103 pedigrees were 599, and the male-to-female ratio was 1.3:1, with a mean age of onset of 43.8 ± 13.0, from 11 to 80. The average duration from the symptom to diagnosis was 56.9 ± 61.6 months. The average duration from symptoms to death or censoring (information traced to Apr, 2015) was 96.7 ± 78.1 months. A median lifespan (age at death or censoring) was 51.5 years. Most patients were autosomal dominant inheritance (94%), just 6 families with autosomal recessive inheritance.

SOD1 analysis was performed in 58 of 103 FALS pedigrees, and 16 known SOD1 mutation types were detected in 19 probands including 15 missense mutations (A4S, C6S, V14M, G16C, G16A, E21G, G41D, H43R, H46R, L84F, G93R, E100G, D101G, S105L, C111Y and 1 deletion mutation (K128 fs X131). TARDBP gene was sequenced in 39 SOD1-negative patients and 4 known missense mutations M337V, S393L, S292N and G348V in exon 6 in 5 kindreds. FUS/TLS gene was also sequenced in SOD1 and TARDBP-negative FALS patients, and 5 different missense mutations (all known R521L, P525L, R524W, R521H and R521G) in exon 15 in 7 kindreds with an autosomal dominant inheritance. C9orf72 repeat expansion was only found in one FALS.

Conclusions: The frequency of gene mutations of SOD1 (18.4%), C9orf72 (1.0%), FUS (6.8%), and TARDBP (4.9%), which was a little higher than that reported in the literature, while the frequency of gene mutations of C9orf72 (1.0%) was much lower. C9orf72 repeat expansion is not major ALS-associated genes in Chinese patients. The survival of FALS cases seems longer, but the male-to-female ratio is lower than reported. Clinical comparison of SOD1, TARDBP, FUS and other FALS patients revealed differences in site of onset (predominantly upper limbs for SOD1, FUS and other FALS and

bulbar for TARDBP mutations), age of onset (older with TARDBP mutations), and in lifespan (shorter for SOD1, FUS mutation). We showed genetic associations with ALS and provide phenotype-genotype correlations with mutation in Chinese patients with FALS.

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P168 GENETIC DIAGNOSIS OF CHINESE PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS USING NEXT GENERATION SEQUENCING

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Keywords: FUS, Chinese, next generation sequencing

Background: To date, more than 23 causative genes have been identified in amyotrophic lateral sclerosis (ALS). Establishing a molecular diagnosis by conventional sequencing is time consuming and infeasible because of the clinical and genetic heterogeneity. Next generation sequencing provides us such an opportunity to make a rapid and correct molecular diagnosis of ALS.

Objectives: We aim to identify the spectrum of mutations in Chinese patients with ALS and further characterize the phenotypes associated with FUS mutations.

Methods: A total of 41 unrelated individuals with ALS were analyzed in current study, including 32 probands from different families and 9 juvenile-onset SALS patients. Several mutations had been previously reported in 8 families (1-2). The remaining 33 patients were analyzed by targeted Next Generation Sequencing (NGS). Twenty-three causative genes of ALS: SOD1; SETX; FUS; ANG; TARDBP; ALS2; FIG4; VAPB; OPTN; DAO; VCP; UBQLN2; SPG11; SIGMAR1; DCTN1; SQSTM1; PFN1; CHMP2B; SS18L1; EWSR1; TAF15; ARHGEF28 and ERBB4 were included in a custom panel.

Results: A total of 19 different pathogenic mutations were found in 22 out of 33 (66.7%) patients after data analysis and sequencing confirmation. Five of the 19 mutations were novel including 3 in FUS, one in ERBB4 and one in DCTN1. The remaining 14 known mutations were located in SOD1, FUS, and TARDBP. Together, in the cohort of 41 patients, 12 patients (29.3%) carried the SOD1 mutations and 7 patients (2 FALS and 5 SALS) (17.1%) carried FUS mutations. The 3 patients with novel FUS mutations had an early-onset age and a relatively rapid disease course. In addition, 3 de novo FUS mutations were found in 3 SALS patients with a juvenile-onset. Cognitive impairment was not observed in this cohort of patients.

Discussion and conclusions: The high accuracy and efficiency of this approach indicate that it could be applied to the diagnosis of FALS and juvenile-onset SALS patients. Our findings have shown that FUS mutations account for a fairly large proportion of ALS and de novo FUS mutations are common in juvenile-onset SALS patients. This suggests that we should perform FUS mutation analysis routinely in juvenile-onset ALS patients even though without a clear family history.

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P169 THE G41D MUTATION IN THE SUPEROXIDE DISMUTASE 1 GENE IS ASSOCIATED WITH SLOW MOTOR NEURON PROGRESSION AND MILD COGNITIVE IMPAIRMENT IN A CHINESE FAMILY WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: ALS, SOD1, mutation

Objectives: To describe a Chinese family with a G41D mutation in superoxide dismutase (*SOD1*) and their clinical phenotypes, including slow motor neuron degeneration and cognitive impairment.

Methods: Exome sequencing was applied using SureSelect All Exon Kit V4 and HiSeq2000. The *SOD1* mutation was confirmed using Sanger sequencing. Neurological examinations and neuropsychological assessments were conducted for all members of this family.

Results: We report the unique genotype of a Chinese family with a heterozygous mutation (G41D) in the superoxide dismutase (*SOD1*) gene. Since 2003, family members have been evaluated at The People's Hospital of Jiangsu Province. Three members, diagnosed with ALS, have the G41D heterozygous mutation and exhibit slow progression of motor neuron function loss as well as mild cognitive impairment. We found that the executive domain, attention domain, language function, calculation tasks, and memory were significantly impaired in these patients compared to healthy family members. All family members in the third generation of this family with G41D heterozygous mutations are younger than 35 years of age and are healthy.

Discussion: This is the first report of a unique disease course in a Chinese family with fALS and a novel G41D mutation in SOD1. Clinical hallmarks of this family suggest late onset (>50 years) and slowly progressing motor neuron symptoms with mild cognitive impairment. Their average survival was longer than 20 years from clinical onset. One family member, the proband's brother, had an earlier onset and relatively rapid lower motor neuron disease progression with cognitive problems. From a clinical point of view, this patient showed a quick progression of disease and died sooner. Alternatively, cognitive problems appear after the upper motor neuron dysfunction has been present for an extended period of time. The only healthy sibling of the proband has wild-type SOD1, strongly supporting the notion that this SOD1 mutation is causative. Other family members harboring this mutation have not yet developed any ALS symptoms; however, this is likely due to their relative youth. In addition to longer survival, other clinical characteristics of our patients suggested cognitive impairments. Mild cognitive deficits occurred during the later stage of disease without significant changes in the frontal and temple regions (based on MRI scans). These clinical features postulate a possible clinical-genetic phenotype of fALS patients with G41D mutations. However, further data are required to establish possible genotype-phenotype correlations.

Conclusions: The G41D mutation in *SOD1* may cause slow progression of motor neuron function loss and cognitive impairment symptoms.

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P170 OPTINEURIN MUTATIONS IN PATIENTS WITH SPORADIC AMYOTROPHIC LATERAL SCLEROSIS IN CHINA

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Keywords: Chinese, optineurin, mutation

Background: Mutations in the optineurin gene (*OPTN*) were first demonstrated to be causative for ALS in 4 Japanese families and 1 SALS patient and exhibit both recessive or dominant inheritance patterns (1). To date, approximately 26 different mutations in *OPTN* have been identified in sporadic and familial ALS patients of Asian and European descent. Mutation frequencies vary substantially among populations. The prevalence of the *OPTN* mutation was particularly high in the Japanese ALS population (3.3%-3.8% in FALS, 0.23%-1% in SALS), whereas in patients of Caucasian descent, few had mutations in *OPTN*. These findings led us to hypothesize whether the *OPTN* gene has a higher incidence among the Asian population. Nevertheless, subsequent studies of South Korean and Taiwanese populations were inconclusive.

Methods: In total, 511 patients with SALS and 204 healthy controls were enrolled in this study. All participants were from Mainland China. Most of the patients had previously been screened for *SOD1*, *TARDBP*, *FUS* and *C9orf72*, and patients with these mutations were not excluded from the study. DNA samples were extracted from peripheral blood obtained from participants with their written informed consent. To detect mutations in *OPTN*, all 13 coding exons were sequenced.

Results: In total, 9 nucleotide variants were identified in this study. Among them, the genetic analysis revealed 4 novel missense mutations (A136V, K395R, I451T and E516Q) that are not contained in the SNP database and have not been previously reported. The SNPs A136V, I451T and E516Q were each present in 1 patient but none of the 204 controls, whereas K395R was found in 1 patient and 1 control participant. All of these mutations were present in a heterozygous state. The remaining 5 detected variants are known SNPs in the SNP database. Two variants (c.408G>A and c.597T>C) had not been linked to ALS. Notably, the patient carrying A136V also harbored the c.597T>C SNP. We did not find a significant difference in the minor allele frequency (MAF) of these 5 SNPs between the patient and control groups. No nonsense mutations were found in our cohort of SALS patients. The A136V and K395R variants were predicted to be benign by the PolyPhen-2 and SIFT bioinformatics programs, whereas the results of the prediction for the I451T and E516Q mutations were inconsistent.

Discussion and conclusions: Our study demonstrates that the prevalence of *OPTN* mutations in SALS patients in China is 0.78% (4/511), which is approximately equivalent to the incidence reported in Japanese and Taiwanese patients (2) but is higher than that of patients in Western countries, confirming its higher prevalence in Asian patients.

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P171 ASSESSMENT OF TREM2 RS75932628 ASSOCIATION WITH AMYOTROPHIC LATERAL SCLEROSIS IN A CHINESE POPULATION

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Keywords: TREM2, rs75932628, genotyping

Background: Although, rs75932628 in triggering receptor expressed on myeloid cells 2 (*TREM2*) was shown to

increase the risk for Alzheimer's disease, there is no agreement on the association between this variant and the risk for amyotrophic lateral sclerosis (ALS).

Methods: We conducted a large-sample study to investigate if this variant is associated with ALS in a Chinese population. A total of 868 sporadic ALS (SALS) and 869 healthy controls were included. All cases were genotyped for the Single Nucleotide Polymorphisms (SNP) using Sequenom iPLEX Assay technology.

Results: The rs75932628-T variant of the *TREM2* gene was not identified in SALS patients and controls.

Conclusions: rs75932628 is unlikely to play a role in the pathogenesis of ALS in Chinese patients with SALS.

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P172 TWO NOVEL MUTATIONS OF DCTN1 IN CHINESE SPORADIC AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Keywords: Chinese, DCTN1,novel mutation

Objective: To date, about 15 novel mutations of *DCTN1* have been reported in familial and sporadic ALS patients, most based on European populations and familial ALS patients. In our study, *DCTN1* was first sequenced in a large Chinese population with sporadic ALS and we analyzed the clinical manifestation and structure changes of different mutations.

Methods: This study included 510 sporadic ALS patients (Male:Female =1.78:1; mean age at examination ± standard deviation (SD) =53 ± 11 years) registered with the Neurological Department of Peking University Third Hospital from 2013 to 2014, and 210 non-neurologic control subjects (117 males, 93 females; Male: Female =1.26:1; mean age at examination ± SD = 55 ± 13 years). All of subjects were of Han ethnicity and the patients met the revised El Escorial criteria of clinically definite, probable, or laboratory-supported probable ALS. All the patients included were under a long-time follow-up for every three months from the enrollment.

Results: We found two heterozygous missense mutations of p.R623W, p.A933V. The patients demonstrated spinal onset; the onset age, and diagnosis delay showed no significant difference. Mutation p.R623W was located in the dynein binding domains, which is important for dynactin function and may have a chance to explain the relationship between *DCTN1* mutation and ALS.

Conclusions: Two novel mutations of *DCTN1* were found in Chinese sporadic ALS patients. Further functional research is needed, familial cases with these mutations would confirm the involvement of *DCTN1* in ALS.

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P173 TUBA4A MAY NOT BE A SIGNIFICANT GENETIC FACTOR IN CHINESE ALS PATIENTS

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Keywords: Chinese population, exome-wide rare variant burden analysis, TUBA4A gene

Background: Recently, it was reported that TUBA4A variations were associated with familial ALS following exome-wide rare variant burden analysis (1). The results of this study identified five deleterious TUBA4A variants (p.T145P, p.R320C, p.R320H, p.A383T, and p.K430N) in 635 familial ALS patients and only three TUBA4A mutations (p.G365E, p.A383T, and p.E386K) in 9,841 controls, all of which occurred within exon 4. Thus, the TUBA4A gene could be a novel ALS-associated gene. Although large cohorts of Caucasian ALS patients have been assessed, studies analyzing Asian ALS patient cohorts have not been reported.

Objectives: In this study, we investigated the occurrence of mutations in the TUBA4A gene in a large cohort of Chinese ALS patients and investigated the relationship between TUB4A mutations and ALS clinical phenotypes.

Methods: This study included 80 FALS pedigrees (47 males, 33 females; mean age of onset ± standard deviation (SD) = 47.7 ± 12.2 years); 500 SALS patients (301 males, 199 females; mean age of onset ± SD = 52.0 ± 11.6 years) and 500 neurologically normal control individuals. All of the patients had previously been screened for the most common mutations in ALS-associated genes, including *SOD1*, *TARDBP*, *FUS, C9ORF72* and *SQSTEM1*.

Results: Only 4 single nucleotide variants have been identified in exon 4 of the TUBA4A gene. Our data confirmed the presence of 1 synonymous variant, c.G528A (p.Q176Q), in a SALS patient. A heterozygous missense mutation, c.G832A (p.A278T), was identified in a healthy control subject, and this change was predicted to have a deleterious effect on the protein product (score=0.04) using the SIFT program (http://sift.bi.a-star.edu.sg/). The heterozygous polymorphism c.A909G (p.V303 V, rs141922502) was identified in a SALS patient and in a control subject.

Discussion and conclusions: In this study, we performed a large case-control analysis of more than 1,000 participants to determine the presence of any associations between mutations in the *TUBA4A* gene and ALS in Chinese patients. Consistent with the most recent exome sequencing data, which did not identify an association between TUBA4A mutations and ALS (2), our study indicates that TUBA4A mutations might not be an associated genetic factor in ALS patients of Chinese descent.

In conclusion, our results are unable to confirm the role of *TUBA4A* mutations in the pathogenesis of ALS in Chinese patients. Therefore, future genetic studies from

different ethnic groups will be required to determine the association between TUBA4A and ALS.

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P174 CHCHD10 WAS IDENTIFIED AS ALS CAUSATIVE GENE WITH COMPLEX MECHANISMS

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Keywords: CHCHD10, mitochondria, sequencing

Background: Exome sequencing combined with traditional linkage analysis and Sanger sequencing offer novel approaches to identify causative genes.

Objectives: To identify novel ALS causative genes.

Methods: 309 familial ALS cases, 84 SALS with disease duration of more than 5 years and 1480 controls were screened for CHCHD10 mutations. Mutation bearing fibroblasts underwent EM, Southern blotting, Western blotting analysis and were examined for mitochondrial respiration and dynamics.

Results: We previously found CHCHD10 mutations in autosomal dominant mitochondrial myopathy. 5 ALS patients from a large pedigree underwent whole genome exome sequencing; CHCHD10 R15L mutation was found and further substantiated with Sanger sequencing and microsatellite genotyping. R15L mutation was found in 4 additional FALS pedigrees, but not in 84 SALS and 1480 controls. One FALS pedigree had homozygous R15L with uniparental disomy.

The five CHCHD10 R15L families had a total of 55 ALS patients and 16 obligate carriers. All 22 ALS patients with onset data had onset of extremities. Average age of onset was at 55 years. Average of disease duration was 81.2 months. One male R15L ALS patient had a muscle biopsy. The whole section had two typical ragged red fibers and two myofibers had NADH-TR positive rimmed vacuoles. Confocal microscopy showed both wild type and R15L CHCHD10 mutant were expressed diffusely in the cytoplasm and neuronal processes, mostly colocalized with mitochondria. Remarkable, tangle like CHCHD10 immunoreactive protein aggregates were present in the cytoplasm and neuritis of FALS spinal cord neurons.

Deletion of amino acid 2-16 did not prevent entry of CHCHD10 into mitochondria. EM of R15L fibroblasts showed mitochondria with swollen body, broken cristae, homogeneous matrix, multivesicular bodies and vacuoles. Southern blotting of mitochondria DNA from R15L fibroblast showed multiple additional bands than controls. Mitochondria DNA copy number by real-time PCR with tRNALeu (UUR) as a mitochondrial probe normalized against nuclear beta-2-microglobulin showed R15L fibroblasts had an average of 1666.6 copies of mitochondria DNA, significantly less than wild type fibroblast with an average of 3687.8 copies. Mitochondria movement was analyzed in 137 mitochondria of 9 motor neurons expressing wild type CHCHD10 and 180 mitochondria of 12 motor neurons expressing R15L CHCHD10. The backward moving frequency of R15L mitochondria was 16.9% ±0.92 (mean ±SEM), compared to 32.2%±4.30 in wild type. R51L fibroblasts showed significantly lower oxygen consumption (OCR) on Seahorse XFe analysis and lower Complex IV and V activity.

Conclusions: The CHCHD10 gene mutation causes ALS with complex mechanisms. Cytoplasmic CHCHD10 protein aggregates were prominent; mitochondrial morphology, DNA stability, respiration and dynamics were also compromised.

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P175 ATAXIN 2 IS NOT A DISEASE MODIFIER IN A LARGE SERIES OF ALS PATIENTS CARRYING THE C9ORF72 EXPANSION

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Keywords: ATXN2, C9ORF72, genetic modifier

Background: The identification of genetic modifiers of amyotrophic lateral sclerosis (ALS) phenotype is a major challenge in ALS. Recently it was reported that *ataxin 2* (*ATXN2*) polyQ intermediate expansions (27) may render *C9ORF72* expansion carriers more susceptible to the development of ALS.

Objectives: To assess the frequency of *ATXN2* intermediate expansions (27-34) in ALS patients carrying *C9ORF72* GGGGCC repeats, compared to ALS patients not carrying *C9ORF72* expansion and matched controls.

Methods: A total of 337 Italian ALS cases (56 of Sardinian ancestry) carrying a *C9ORF72* expansion were assessed in this study. Cases were identified through ITALSGEN, a collaborative consortium of 18 Italian ALS centers. *C9ORF72* cases were compared to 1640 ALS cases non-mutated for C9ORF72 (299 Sardinian) and 1043 (243 Sardinian) ethnically matched controls.

Results: Intermediate *ATXN2* expansions were detected in 6 *C9ORF72* ALS cases (1.8%), 89 non-*C9ORF72* cases (5.4%) and 34 controls (3.3%). The largest alleles observed in *C9ORF72* cases were 30 (1 case) and 29 (2 cases). The frequency of *ATXN2* polyQ intermediate expansions in *C9ORF72* cases was lower but not significantly different from that of controls (p=0.111) and lower than in non-*C9ORF72* patients (p=0.002). *ATXN2* polyQ intermediate expansions were more frequent in non-C9ORF72 patients than in controls (p=0.006).

Discussion: In this large series of Italian ALS patients, *ATXN2* polyQ intermediate expansions were not a genetic modifier of ALS related to C9ORF72. Conversely, *ATXN2* polyQ intermediate expansions are significantly more common in non-C9ORF72 ALS patients than in matched controls.

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P176 EXOME SEQUENCING REVEALS NOVEL TBK1 MUTATION AND RARE TUBA4A VARIANT IN FAMILIAL ALS

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Keywords: familial ALS, TBK1, TUBA4A

Background: Mutations in major amyotrophic lateral sclerosis (ALS) genes, including superoxide dismutase-1 (*SOD1*), TAR DNA-binding protein (*TARDBP*), fused in sarcoma (*FUS*), and chromosome 9 open reading frame 72 (*C9ORF72*), explain approximately 50% of familial ALS (fALS). Hence, although considerable efforts have been put into gene discovery, a large proportion of fALS remains unexplained.

Objectives: To characterize a cohort of fALS patients without mutations in major ALS genes and to discover variants accounting for their disease.

Methods: Exome sequencing was performed on a cohort of 24 fALS patients who did not carry mutations in *SOD1*, *TARDBP*, or *FUS*, or a *C9ORF72* repeat expansion. DNA was processed at the Mayo Clinic Medical Genome Facility using the SureSelect Human All Exon V4 plus UTR kit (Agilent Technologies). Sequencing was performed on a HiSeq 2000 system (Illumina) with three barcoded samples per sequencing lane, generating 101-base paired-end reads. A web-based software tool (ie GEnomes Management Application (GEM.app)) was used to analyze genomic variant data. Analysis was restricted to a list of 142 genes possibly implicated in ALS pathogenesis based on public databases (eg Amyotrophic Lateral Sclerosis Online genetics Database (ALSoD)) and current literature. Additional filters were used, including predicted pathogenicity by at least one in silico software application, read depth above 10x, genotype quality score over 50, and no reports in NHLBI Exome Variant Server (EVS) or Haplotype Map (HapMap) databases. Identified variants were confirmed through Sanger sequencing.

Results: A total of 18 variants were discovered using aforementioned filters; all of which were validated by Sanger sequencing. Variants were present in 16 different genes, accounting for over 50% of our fALS patients. Of interest, these variants included a novel TANK-binding kinase 1 (*TBK1*) mutation (p.L717S) and a rare tubulin, alpha 4a (*TUBA4A*) variant (p.I422F). Both variants were predicted to be deleterious and well conserved. The novel *TBK1* mutation was detected in a female with bulbar onset ALS starting in her fifties. This mutation, located in exon 21, has not been reported in any public database. The *TUBA4A* variant was observed in a female who developed limb onset ALS in her forties; it is located in exon 4 and has only been reported once in the Exome Aggregation Consortium (ExAC) database.

Discussion and conclusion: Exome sequencing of our fALS cohort uncovered 18 variants, including a novel *TBK1* mutation and a rare *TUBA4A* variant. The *TBK1* and *TUBA4A* variants were each encountered in a single patient, predicted to have deleterious effects, and are well conserved. *TBK1* and *TUBA4A* have recently been associated with ALS, and are involved in autophagy and cytoskeletal stability. Our findings, thus, confirm that *TBK1* and *TUBA4A* are implicated in ALS, and stress the crucial role of the autophagy-lysosomal pathway in its pathogenesis.

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P177 ANALYSES OF THE VCP GENE IN PATIENTS WITH SPORADIC AMYOTROPHIC LATERAL SCLEROSIS, IDENTIFY A NOVEL MUTATION ASSOCIATED WITH INCREASED SUSCEPTIBILITY TO OXIDATIVE STRESS

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Keywords: frontotemporal dementia (FTD), sporadic, oxidative stress

Background: Mutations in the *VCP* gene encoding valosin-containing protein (VCP) cause inclusion body myopathy with Paget's disease of the bone and frontotemporal dementia (IBMPFD) and amyotrophic lateral sclerosis (ALS). A recent study from China failed to identify any mutations in this gene in 324 patients with sporadic ALS.

Methods: We sequenced the *VCP* gene in 90 Japanese patients with sporadic ALS, who were negative for mutations in other genes causative for ALS. Cultured fibroblasts from a mutation-positive patient was treated with l-buthionine sulfoximine (BSO), an oxidative stress-inducer that inhibits synthesis of glutathione, a free-radical scavenger. SH-SY5Y cells was transiently transfected with plasmids encoding wild-type (Wt) or mutant VCP, were also treated with BSO.

Results: Our analyses identified a novel VCP mutation, p.Arg487His in a patient. This patient felt bilateral weakness of the proximal upper limbs at the age of 61 years. He had no family history of neuromuscular disease, but a detailed history revealed that his elder brother (patient 2) had dementia, which was later found to be frontotemporal dementia associated with typical imaging findings of the brain.

Patient 2 also had Parkinsonism, a condition occasionally found in patients with VCP mutations. Unfortunately, he had already died, and no DNA or further detailed clinical information was available. Patient 1 visited our hospital at the age of 65 years. Neurologic examinations revealed bulbar signs and weakness with severe amyotrophy of all four limbs. Deep tendon reflexes were diminished, but extensor plantar reflexes were present. He soon required a percutaneous endoscopic gastrostomy (PEG) tube and 1 month later artificial ventilation through a tracheostomy tube at the age of 66 years.

Higher functions and personality were apparently normal until the age of 69 years. He had difficulty in using a computer at 70 years of age, because he tended to forget the places of words in the communication method. Since then, his personality was mildly changed with decreased attention. Fibroblasts from this patient was susceptible to BSO-induced oxidative stress. Transient expression of the newly identified mutant as well as of known mutants also rendered SH-SY5Y neuroblastoma cells vulnerable to oxidative stress.

Discussion and conclusion: The presence of the mutation in the Japanese population extends the geographic region for involvement of the VCP gene in sporadic ALS to East Asia. In addition, our findings suggest the involvement of oxidative stress in the pathomechanism of VCP-related ALS.

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P178 VALOSING-CONTAINING PROTEIN (VCP) GENE ANALYSIS IN A COHORT OF ALS PATIENTS: IDENTIFICATION OF A NOVEL MUTATION

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Keywords: VCP gene, genetics, clinics

Background: Amyotrophic lateral sclerosis (ALS) is a complex disease due to genetic and environmental factors. In 10% of cases the disease is familiar (FALS) while in the remaining cases it occurs sporadically (SALS). To date more than 20 different genes are disease causative, the most common being SOD1, FUS, TARDBP and C9ORF72.

Objectives: To determine the presence of valosin-containing protein (VCP) gene mutations in a cohort of 103 Italian ALS patients (9 FALS and 94 SALS). Mutations in VCP) gene accounts 1% of FALS cases, however mutations in this gene have been found in other disorders frequently associated with ALS such as cognitive impairment, dementia, myopathy and Paget's disease.

Methods: ALS patients were enrolled at the NeuroMuscular Ominicentre (NEMO) and Neurological Clinic according to El Escorial criteria. Genetic counselling was offered by Medical Genetics Unit together with molecular analysis for SOD1, TARDBP, FUS, C9ORF72

and VCP genes. Genomic DNA extraction, PCR amplifications and purifications were performed on an automated Beckman Coulter platforms. All probands were amplified and directly sequenced for all exons of SOD1, exon 6 of TARDBP, exons 5, 6, 13, 14 and 15 of FUS and exons 2, 3, 5, 6, 10, 14 of VCP gene. Direct DNA sequencing included coding regions as well as splice site regions. Repeat-primed PCR assay was applied in order to screen for the presence of the GGGGCC hexanucleotide repeats expansion in C9ORF72 gene.

Results: Screening of 109 ALS subjects led to the identification of one VCP mutation carrier. This mutation was not previously reported in literature or through different prediction softwares (Mutation Taster, Polyphen and SIFT). Patients with the mutation were affected by Paget's disease, which subsequently evolved in classic ALS. Overall we screened 103 ALS patients: 53 female and 50 Male .

Discussion and conclusion: In our study VCP gene mutations are confirmed as a rare cause of ALS disease even when there is a coexistence of cognitive impairment, dementia, myopathy and Paget's disease.

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P179 A RAPID FUNCTIONAL DECLINE TYPE OF SPORADIC ALS IS LINKED TO LOW EXPRESSION OF TTN

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Keywords: functional decline, SNPs

Objective: To classify the patterns of functional decline in patients with sporadic amyotrophic lateral sclerosis (ALS) and explore the genetic backgrounds that modified these patterns.

Methods: We included 465 patients with sporadic ALS in the analysis and clustered the longitudinal functional scores in the registered patients using a mixture approach of the nonlinear mixed-effects model. We conducted a genome-wide analysis of 572,983 single nucleotide polymorphisms (SNPs). We then assessed the association between the clusters of longitudinal functional scores and SNPs.

Results: We identified the following 4 clusters of longitudinal functional decline in the cases: a rapid functional decline cluster; an intermediate functional decline cluster; a sigmoidal functional decline cluster and a moderate functional decline cluster. We identified 7 SNPs associated with the rapid functional decline cluster using a recessive model ($P=3.47\times10^{-8}$ 8.34×10^{-8}). The odds ratio for the probabilities of the rapid decline cluster ranged 5.5 to 5.84. Homozygosity for the minor alleles in the 7 SNPs, which constituted a linkage disequilibrium (LD) block, was associated with decreased expression of TTN (encoding Titin, a large sarcomere protein) in the expression quantitative trait loci database of a large-scale Japanese genetic variation database ($P=8.6\times10^{-10}$ 1.1×10^{-7}). TTN expression in immortalised lymphocyte lines was decreased in patients who were homozygous for the minor alleles compared with those who were homozygous for the major alleles (n=19 in each group, P=0.002).

Conclusion: We detected an LD block associated with a rapid functional decline in sporadic ALS patients, which is linked to decreased expression of TTN. Titin may serve as a therapeutic target to ameliorate the progression of sporadic ALS.

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P180 WHOLE EXOME SEQUENCING IN SPORADIC ALS TRIOS AND FAMILIAL ALS

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Keywords: whole exome sequencing, mutations, trios

Background: Whole exome sequencing (WES) has become a new means of identifying novel and previously known disease mutations in coding regions of the human genome.

Objectives: We used WES in patient-parent trios and familial ALS kindreds to identify new genes associated with sporadic and familial ALS.

Methods: DNA samples from 50 patient-parent trios with sporadic ALS, and two recessive ALS families were prepared using the Agilent SureSelect 70 MB kit and sequenced in Illumina HiSeq2500. The data were analyzed using a High Power Computing cluster built in-house. Open source bioinformatics tools such as BWA, SAMtools, GATK, Picard, and VCF, as well as, in-house

developed scripts and analytic models were used to identify novel variants.

Results: Statistical analysis of the coverage showed that over 99% of the exons were covered in most of the samples with average depth of 120x (range per sample was 70x-210x). An average of 550,000 to 750,000 variants per sample were generated. After filtering them further, 400-700 novel variants were identified in the coding region. Subsequently, we focused on identifying common variants among all of the samples. The common variants were verified using conventional Sanger sequencing. Furthermore, to verify the statistical importance of these mutations mathematical modeling in logistic regression analysis was used to identify nuclear, mitochondrial and RNA pathways, which may have direct impact on the disease. We next performed immunohistochemistry on autopsy tissue samples from patients to explore the involvement of the identified genes/proteins in disease pathogenesis. *In-vitro* and *in-vivo* cell modeling are currently underway to verify the functionality of identified mutations.

Discussion and conclusions: WES is a powerful tool that has allowed us to identify mutations in both familial and sporadic cases of neurodegenerative diseases.

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P181 THE ROLE OF DE NOVO MUTATIONS IN THE DEVELOPMENT OF SPORADIC ALS

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Keywords: de novo mutations, whole genome sequencing

Background: Since the majority of ALS cases appear to be sporadic, the occurrence of *de novo* mutations in the germline of an unaffected parent might be an important mechanism in its causation.

Objective: We set out to test whether the rate of *de novo* mutations is increased in ALS and if *de novo* mutations can point us in the direction of genes or pathways that are important for the development of ALS in general.

Methods: Using whole-genome sequencing we identified *de novo* mutations in 21 trios (healthy parents and affected patient: 63 sequenced genomes in total). We combined these results with previous published *de novo* mutations in ALS (1-2) and investigated the nature of these mutations.

Results: We discovered 15 new *de novo* mutations in the 21 trios, a number that is not increased based on known mutation rates per generation. These, and previously published *de novo* mutations in ALS occur in genes that already have a significant higher *de novo* mutation rate in the general population (p=5.4x10-9) and show no specific protein-protein interaction. Adding 39 whole-genome sequenced familial ALS patients revealed a significant enrichment of mutations in *PXDNL*, compared to the Exome Sequencing Project (n=4300) and GoNL (n=498) datasets (p=0.02). In addition, we found a cellular phenotype for mutations in an isoform of *PXDNL*. To validate these findings, we performed a large next generation targeted sequencing experiment with 1011 sporadic ALS patients and 2022 matched controls that showed no increased mutational burden in PXDNL (p=0.09). Importantly, mutational burden in our matched controls was statistically different from the controls from the Exome Sequencing Project (p=0.0004) and the Exome Aggregation Consortium (n=33370, $p < 0.00001$).

Conclusions: This study combined previous and new data on *de novo* mutations in ALS and shows a limited, if any, role for *de novo* mutations in this disease. The study clearly illustrates the significant differences between allele frequencies derived from controls and publicly available datasets, emphasizing the importance of including controls that are adequately matched to the cases.

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P182 IDENTIFICATION OF DE NOVO MUTATIONS IN SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: exome sequencing, trios, de novo mutations

Background: It is believed that the heritability of sporadic amyotrophic lateral sclerosis (SALS) is in large part due to rare genetic variants that have mostly eluded identification using conventional genetic approaches. Interestingly, de novo mutations (DNMs) have been shown to explain a large part of heritability of complex neurological diseases such as schizophrenia, autism, and sporadic mental retardation. More recently, DNMs have been identified in ALS patients, suggesting a possible role in the pathogenesis of ALS (1,2).

Objectives: To confirm and expand the possible contribution of DNMs to SALS susceptibility, using a trio-based exome sequencing approach.

Methods: We performed exome sequencing in 39 Italian SALS trios, each one composed of a patient and his/her unaffected parents. After filtering, candidate DNMs were validated by Sanger sequencing, and existing exome databases were interrogated for additional mutations in DNM-containing genes. *In silico* network analysis was performed to identify biological pathways enriched for SALS-associated DNMs.

Results: We identified 32 non-synonymous missense DNMs in 26 trios (1.2 variant/exome). Four variants were already annotated in public databases, while the remaining 28 were novel. No DNM occurred in more than one instance, neither did we observe multiple DNMs in the same gene. Case-control analysis did not reveal a significant association of DNM-containing genes with FALS and SALS susceptibility. Conversely, pathway analysis revealed an enrichment of genes involved in calcium ion transport (p=0.023), and in purine nucleoside binding (p=0.037). By incorporating the results of previous studies on ALS trios (1, 2), we could also confirm the overrepresentation of DNMs in genes involved in transcriptional regulation (p=0.001) and chromatin modification (p=0.003).

Discussion and conclusions: Since all identified variants are private and in genes not previously associated to ALS, establishing the individual contribution of each DNM to the disease is not possible. Despite this limitation, our study confirms the occurrence of DNMs in SALS and suggests that rare variants in genes involved in calcium ion transport and transcriptional regulation contribute to ALS pathogenesis.

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P183 DE NOVO MUTATIONS OF THE FUS GENE ARE THE MOST FREQUENT CAUSE OF SPORADIC ALS IN VERY YOUNG ONSET PATIENTS

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Keywords: early-onset, fused in sarcoma (FUS), de novo mutation

Background: In ALS patients with a known genetic cause, mutations in *C9orf72* and *SOD1* account for the majority of familial and late-onset sporadic cases, whereas mutations in *FUS* can be identified in just around 5% of familial and 1% of overall sporadic cases (1–2). There are only few and merely anecdotic reports on *de novo FUS* mutations in juvenile ALS patients (3–7). To date, no systematic evaluation on the frequency of *de novo FUS* mutations in early-onset ALS patients has been conducted.

Objectives: The aim of this study was to systematically investigate the incidence of *de novo FUS* mutations in "true" sporadic, i.e. confirmed by genetic testing of the parents, very young ALS patients.

Methods: We screened a cohort of 14 early-onset sporadic ALS patients (onset age < 35 years) to determine the frequency of mutations in *C9orf72*, *SOD1* and *FUS* in this defined patient cohort. All patients were recruited prospectively by a single center over a period of 38 months.

Results: No mutations were detected in *SOD1* or *C9orf72*, however we identified six individuals (43%) carrying a heterozygous *FUS* mutation including one mutation that has not been described before (c.1504delG (p.Asp502Thrfs*27). Genetic testing of parents was possible in five families and revealed that the mutations in these patients arose *de novo*. Three of the six identified patients presented with initial bulbar symptoms.

Discussion and conclusion: Our study identifies *FUS* mutations as the most frequent genetic cause in early-

onset ALS. Genetic testing of *FUS* thus seems indicated in sporadic early-onset ALS patients especially if showing predominant bulbar symptoms and an aggressive disease course.

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Theme 10 Molecular Cell Biology

P184 SUBCELLULAR LOCALIZATION AND RNAS DETERMINE FUS ARCHITECTURE IN DIFFERENT CELLULAR COMPARTMENTS

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Keywords: fused in sarcoma, RNA, subcellular localization

Mutations in Fused in sarcoma (FUS) gene cause a subset of familial amyotrophic lateral sclerosis (ALS), a fatal motor neuron degenerative disease. Wild-type FUS is largely localized in the nucleus, but mutant FUS accumulates in the cytoplasm and forms inclusions. It is unclear whether FUS depletion from the nucleus or FUS inclusions in the cytoplasm triggers motor neuron degeneration.

In this study, we revealed the nuclear and cytoplasmic FUS proteins form distinct local distribution patterns. The nuclear FUS forms oligomers and appears granular under confocal microscope. In contrast, the cytoplasmic FUS forms inclusions with no oligomers detected. These patterns are determined by the subcellular localization of FUS, regardless of wild-type or mutant protein. Moreover, mutant FUS remained or re-directed in the nucleus can oligomerize and behave similarly to the wild-type FUS protein. We further found that nuclear RNAs are critical to its oligomerization.

Interestingly, the formation of cytoplasmic FUS inclusions is also dependent on RNA binding. Since the ALS mutations disrupt the nuclear localization sequence, mutant FUS is likely retained in the cytoplasm after translation and interacts with cytoplasmic RNAs. We therefore propose that local RNA molecules interacting with the FUS protein in different subcellular compartments play a fundamental role in determining FUS protein architecture and function.

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P185 THE PHOSPHORYLATION OF FUS BY CK2 REGULATES ITS RNA-BINDING ACTIVITY

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Keywords: FUS, phosphorylation, RNA binding

Mutations on a RNA-binding protein named FUS (fused in sarcoma) are related to an inherited form of the neurodegenerative disease amyotrophic lateral sclerosis (ALS). FUS positive cytoplasmic inclusions are commonly observed in these patients. Altered RNA metabolism is increasingly implicated in ALS, however the pathogenic mechanism underlying the mutant FUS-caused familial ALS is unknown. Various protein modifications including phosphorylation on FUS have been found to be involved in regulating FUS function.

In this study, we found that FUS contains casein kinase 2 (CK2) consensus phosphorylation sequence near its RNA recognition motif (RRM). We also demonstrated that FUS can be phosphorylated by CK2 both *in vitro* and *in vivo*.

Functionally, phosphorylation-mimetic mutants of FUS on the CK2 sites showed a stronger RNA binding activity as compared to the wild-type FUS. Moreover, the ALS-associated R521G mutant FUS was triggered to form more and larger cytoplasmic inclusions when phosphorylated by CK2. Taken together, CK2-mediated FUS phosphorylation regulates both RNA-binding and cytoplasmic inclusion formation, playing an important role in FUS-related familial ALS mechanism.

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P186 THE EFFECTS OF STERIC HINDRANCE ON RNA EDITING USING ANTISENSE OLIGONUCLEOTIDES

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Keywords: calcium signalling, neurodegeneration

Background: AMPA receptors (AMPARs) are a subset of ionotropic glutamate receptor composed of one or more of four subunits (GluA1-4). AMPARs play a central role in normal and abnormal synaptic function within the nervous system and are permeable to calcium unless the GluA2 subunit is present. GluA2 subunits undergo RNA editing at a specific adenosine, resulting in a change in amino acid residue from glutamine to arginine, critical for regulating calcium permeability. RNA editing is performed by a family of enzymes called Adenosine Deaminases Acting on RNAs (ADARs) and is highly dependent on the double-stranded structure of the RNA transcript. RNA editing in AMPAR subunits is inefficient in patients with Amyotrophic Lateral Sclerosis (1). RNA editing can be prevented by disruption of secondary structure in the RNA molecule using antisense oligonucleotides (ASOs), as previously described (2). However,

there is little information on how targeted disruption of RNA editing can influence cell viability and neuronal cell death.

Objectives: To manipulate RNA editing of AMPAR subunits using ASOs through disruption of the GluA2 double-stranded RNA structure.

Methods: ASOs were transfected into HeLa cells along with a small section of the GluA2 subunit (n=6 per treatment group). Editing was quantified by a RT-PCR based assay on RNA extracts followed by densiometric analysis of BbvI digestion products.

Results: A novel ASO was designed to target the Q/R site of the GluA2 subunit and was compared to the previously published ASO. HeLa cells were treated with 10 μM of each 25mer ASOs targeting the GluA2 RNA transcript. The previously published ASO reduced editing to 70.1 1.44% of control, while the newly designed ASO reduced editing to 25.9 0.69% compared to control.

Discussion: A new ASO has been designed that shows an improved reduction in RNA editing at the Q/R site compared to a previously published sequence. This ASO will now be tested in primary neuronal cultures in order to assess the impact of impaired RNA editing on cell viability.

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P187 IDENTIFICATION OF NOVEL SUBSTRATES OF THE ALS-ASSOCIATED RIBONUCLEASE ANGIOGENIN VIA RNA SEQUENCING

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Keywords: angiogenin, RNA, translation

Background: Angiogenin is a secreted member of the pancreatic ribonuclease A superfamily. ALS-associated loss-of-function point mutations have been identified in both familial and sporadic forms of the disease. Angiogenin is a stress-induced protein expressed and secreted from motorneurons, whereupon it is endocytosed by neighbouring glia cells (1). Angiogenin treated astrocytoma cells show cleavage of tRNAs however a systematic analysis of Angiogenin substrates has not yet been performed.

Objectives: We aimed to identify novel substrates of the ALS-associated ribonuclease Angiogenin using our paracrine model of stress-induced signaling.

Methods: Previous work in our group identified Angiogenin as a stress-inducible secreted protein which is endocytosed by neighbouring astrocytes in mouse primary mixed motorneuron cultures. To determine the role of angiogenin in astrocytes we use a human cell line model to mimic this paracrine interaction. MZ-294 human astrocytoma cells are treated with recombinant human angiogenin (R&D systems) in serum-free media. Under these conditions MZ-294 cells internalise angiogenin where it induces RNA cleavage. RNA sequencing was performed on small RNAs isolated from Angiogenin-treated cells and cleavage products were identified as differentially expressed RNA fragments. Angiogenin-induced RNA cleavage products were validated by northern blotting and custom Taqman assays.

Results: We have identified numerous highly structured non-coding RNAs that are cleaved by Angiogenin. Numerous tRNA fragments were detected from a range of different tRNA isotypes and isoacceptors however 5' tiRNAs were more abundant than 3' tiRNAs. The 5' tiRNA fragments were validated by northern blotting and were absent in cells treated with a catalytically inactive angiogenin mutant (K40I). In addition to tRNA fragments numerous novel substrates were identified comprising functional non-coding RNAs including snoRNAs and snRNAs, amongst others.

Discussion and conclusions: We demonstrate here that in addition to the well characterised role of Angiogenin in tRNA cleavage during stress, Angiogenin cleaves a novel set of functional non-coding RNAs. Several of the novel Angiogenin cleavage products are involved in translation-associated processes indicating that Angiogenin may act via multiple pathways to suppress protein translation during cellular stress.

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P188 UBIQUILIN2 PROTEIN INTERACTS WITH HNRPN FAMILY THROUGH PXX DOMAIN AND HNRNPA1 AGGREGATES IN SPORADIC ALS PATIENTS

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Keywords: ubiquilin2, protein interaction

Background: Mutations in Ubiquilin2 cause X-linked juvenile and adult-onset ALS and ALS/dementia. Previous studies *in vitro* and *in vivo* showed the ubiquilin2 mutations cause proteasome dysfunction. Ubiquilin2 contains at least four functional domains. Ubiquilin2 binds to the proteasome system with its UBL (ubiquitin-like) domain at N-terminus, and with other ubiquinated proteins through its UBA (ubiquitin associated) domain at C-terminus. It has four STI1 domains and a proline repeat domain (PXX) for interaction with other proteins. Interestingly, mutations related with ALS and ALS/dementia are mapped in or near the proline repeat domain, including the five mutations initially reported by our group. In order to further understand the binding partners of ubiquilin2, different ubiquilin2 domains were engineered individually or in combination into a flag-tagged system.

Objectives: Identify ubiquilin2 protein binding patterns using mass spectometry.

Methods: The flag-tagged PXX domain (AA463-AA581) truncated protein was expressed in neuro2A cells, and immunoprecipitated by anti-flag antibody, resulting cell lysates were analysed using mass spectometry.

Results: HnRNP family members were pulled down and analysed by mass spectrum, as found previously these included hnRNPA1, and hnRNPA2/B and hnRNPU. To further investigate the role of hnRNPA1 in protein aggregation, we immunostained spinal cords from sporadic ALS patients with antibodies against hnRNPA1 and found pathogenic hallmark of skein-like inclusions decorated with anti-hnRNPA1 stains. This experiment provides a direct link between Ubiquilin2 and hnRNPA1.Our studies show that Ubiquilin2 may be an important component in modulating the aggregation of ALS causative proteins.

Conclusion: This interaction between Ubiquilin 2 with RNA binding proteins, suggests that Ubiquilin2 may play a role in linking the protein degradation of those proteins. As a result, mutant Ubiquilin2 may cause their aggregation.

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P189 NEW FUNCTION OF SUPEROXIDE DISMUTASE 1 IN THE NUCLEAR COMPARTMENT

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Keywords: SOD1, nuclear compartment, DNA damage

Background: Recent evidence shows that in PBMCs from sALS patients there is an over-expression of SOD1 mRNA (1), which is in contrast with the unchanged cytoplasmic level of the protein (2). This discrepancy could be explained by a re-localization of the missing protein in the nucleus. Moreover, in PBMCs of sALS patients, SOD1 translocates from the cytoplasm to the nuclear compartment in stressful conditions (3). In yeast, it has also been suggested that SOD1 has a new function in controlling the oxidative stress response (4).

Objectives: The exact role of SOD1 in the nuclear compartment remains a critical issue to clarify. Thus, we aim to investigate whether and how nuclear SOD1 could act against oxidative stress, both in the neuroblastoma cell line SHSY5Y (a cellular model of neurodegeneration) and in PBMCs of sALS patients.

Methods: SOD1 localization in SHSY5Y and in sALS patients was investigated by both western blot and immunofluorescence. Using Mass Spectrometry (MS) we searched for modifications that would allow for SOD1 translocation and immunoprecipitation (IP) and we identified the binding protein involved in regulation of SOD1 localization. Finally, the protective role of nuclear SOD1 towards DNA damage was investigated using the Comet assay.

Results: First, we confirmed that under oxidative stress SOD1 re-locates into the nucleus; SOD1 levels decreases after 30 min of 1mM H_2O_2 treatment, and rescues at T60; probably as a consequence of new protein synthesis (5). MS data highlight that SOD1 nuclear re-localization is prompted by phosphorylation of both serine and threonine at T60. Moreover, the kinase enzyme Chk2 seems to play a critical role in the regulation of SOD1 localization. Comet assay revealed that SOD1-NLS cells showed no comets, indicating that, when SOD1 is located into the nucleus, minor or none DNA fragmentation occurred. Finally, an increase in histone H3 acetylation at both T30 and T60, suggested a possible involvement of nuclear SOD1 in gene transcription.

Discussion and conclusion: In response to oxidative stress, we demonstrated that SOD1 re-locates to the nucleus, where it displays new functions. We reported an involvement of nuclear SOD1 in providing resistance against oxidative DNA damage, and a role in the regulation of gene transcription.

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P190 GENOME-WIDE EXPRESSION STUDY IDENTIFIES HOMEOBOX GENES AND TRANSTHYRETIN IN C9ORF72 EXPANSION CARRIERS

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Keywords: C9ORF72, transthyretin, homeobox genes

Background: The most common genetic cause of frontotemporal dementia (FTD) and motor neuron disease (MND) is a hexanucleotide repeat expansion in chromosome 9 open reading frame 72 (*C9ORF72*). The pathogenic mechanisms underlying *C9ORF72*-related diseases remain largely unknown, and no validated biomarkers currently exist to diagnose these diseases, to predict disease progression, or to monitor the effects of potential treatments.

Objective: To uncover the mechanisms underlying *C9ORF72*-related diseases and to identify potential biomarkers, we performed the first genome-wide brain expression study in *C9ORF72* expansion carriers.

Methods: Our study cohort comprised 32 *C9ORF72* expansion carriers, 30 FTD or MND patients without these expansions, and 20 control subjects without neurological diseases for whom cerebellum and frontal cortex were available. These individuals were selected for a genome-wide expression analysis using Whole-Genome DASL HT Assays (Illumina).

Results: Interestingly, we detected a significant cerebellar up-regulation of homeobox (*HOX*) genes in *C9ORF72* expansion carriers (eg homeobox A5 (*HOXA5*); p-value=4.1e-14). Pathway analysis aligned with these findings and demonstrated enrichment for developmental processes (e.g. skeletal system development; p-value=4.1e-09). Moreover, we noticed a significant cerebellar up-regulation of transthyretin (*TTR*; p-value=5.0e-04). In the frontal cortex, only three genes were differentially expressed, including *HOX* genes and *C9ORF72*. The cerebellar up-regulation of *HOXA5* and *TTR* was validated using quantitative real-time PCR, which revealed specificity for *C9ORF72*-related diseases: expression levels were significantly higher in our expansion carriers as compared to FTD and MND patients without these expansions, to Alzheimers disease patients, to progressive supranuclear palsy patients, and to controls without neurological diseases (p-value$\leq$0.002). *In vitro* experiments demonstrated that loss of *C9ORF72* expression resulted in *HOXA5* and *TTR* up-regulation (p-value$\leq$3.3e-07), whereas their expression was not significantly affected by transfections with expression vectors in the wild-type or expanded range. This indicates that the loss of *C9ORF72* expression encountered in *C9ORF72* expansion carriers might trigger the increase in *HOXA5* and *TTR*. Furthermore, we showed that changes in RNA levels of *TTR* were reflected by changes in its protein levels in the cerebellum, plasma, and cerebrospinal fluid (CSF).

Discussion and conclusions: We report cerebellar up-regulation of *HOX* genes and *TTR* in *C9ORF72* expansion carriers. Our identification of genes involved in developmental processes, points towards compensatory mechanisms that influence the occurrence, presentation and/or progression of *C9ORF72*-related diseases. Moreover, given TTRs neuroprotective effects and that it can be detected in both plasma and CSF, we postulate that it may serve as a biomarker for *C9ORF72*-related diseases and that it could help to uncover biomarker patterns in sporadic diseases. Our findings, therefore, increase our understanding of *C9ORF72*-related diseases, and additionally, reveal a potential biomarker and interesting targets for novel treatment strategies.

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P191 C9ORF72 REPEAT EXPANSIONS DYSREGULATE NETO1 IN FTD-ALS

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Keywords: NETO1, C9ORF72, Frontotemporal degeneration

Background: Frontotemporal degeneration (FTD) is the second most common presenile dementia of individuals under the age of 65. Even though clinically distinct from each other, it has been known that a link between FTD and ALS exists, resulting in some patients developing an overlap syndrome with features of both disorders. One of the most recent advances of FTD-ALS was the discovery that a large percentage of sporadic and familial ALS and FTD and familial FTD-ALS carry a hexanucleotide repeat expansion $(GGGGCC)_n$ in the first intron of C9ORF72, the exact role of which remains unknown. We hypothesize that C9ORF72 expansions alter specific downstream targets involved in mediating neurodegeneration, and have focused our studies on a target involved in glutamate regulation, NETO1.

Objective: The objectives of this study were to: (i) identify targets differentially regulated in $C9^+$ disease versus $C9^-$ disease states; (ii) determine if misregulation of these targets lead to functional consequences.

Methods: We subjected 3 $C9^+$ and 3 $C9^-$ patient B-lymphocyte cell lines, obtained from a public biorepository, to microarray analysis. Following statistical identification of differentially regulated targets, we confirmed specific hits via qPCR. For confirmation of the

relationship between repeats and target transcripts, we transfected astrocytes with a plasmid containing $(C_4G_2)_{12}$, or control, and assessed the effect on NETO1 protein through western blot analysis and RNA expression via qPCR.

Results: Microarray analysis of $C9^+$ and $C9^-$ B-lymphocytes identified NETO1 as being significantly upregulated in $C9^+$ patients ($p<0.01$). This was confirmed by qPCR (600-fold, $p<0.01$). An increase in protein was also observed. We confirmed dysregulation of NETO1 in iPS–motor neurons derived from $C9^+$ patients as NETO1 is a CNS protein. Importantly, introduction of $C9^+$ repeats into astrocytes was sufficient to increase NETO1 RNA levels by 1.6-fold ($p<0.05$). We identified a putative miRNA regulatory site within the regulatory region of NETO1's, and asked whether the predicted miR binding partner could regulate NETO1. In fact, introduction of this particular miR was sufficient to decrease NETO1 by 0.6-fold, ($p<0.01$). In separate studies, we have independently shown that this miR is functionally impaired in C9+ cells.

Discussion and conclusion: The results of these studies demonstrate expanded repeats in $C9^+$ cells increase NETO1 expression at both the mRNA and protein level. This is intriguing since NETO1 has been implicated in glutatmate receptor subunit regulation and glutamate excitotoxicity is a major functional consequence of the disease process. We are currently exploring whether NETO1 upregulation contributes independently to glutamate excitotoxicity. Furthermore, we have identified a miR that is functionally downregulated in $C9^+$ cells and predicted to bind NETO1. Taken together with the results of this study, we hypothesize that this miR's downregulation in $C9^+$ cells leads to diminished binding to NETO1, resulting in the consequential upregulation of NETO1.

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P192 C9ORF72 HAPLOINSUFFICIENCY LEADS TO COMPROMISED RAB29 MEDIATED EXTRACELLULAR VESICLE SECRETION, TRANS-GOLGI TRAFFICKING AND BASAL AUTOPHAGY IN C9ORF72-ASSOCIATED ALS AND FTD

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Keywords: C9ORF72, extracellular vesicles, antisense oligonucleotides

Background: A $(GGGGCC)_n$ hexanucleotide repeat expansion in intron 1 of the C9orf72 gene is the most common cause of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar dementia (FTLD), in both sporadic and familial patients. The pathological mechanism of the disease is largely unknown, including the role and function of C9orf72 itself in cellular pathways. Recent data has shown a link between C9orf72-related ALS and the dysregulation of endosomal trafficking pathways.

Methods: C9/ALS patient-derived fibroblasts, SHSY5 neuroblastoma cell lines and iPS-motor neurons were used to model the disease phenotypes of C9orf72-related ALS. Extracellular vesicle (EV) secretion was examined using nanoparticle tracking analysis. EVs were isolated via a combination of ultracentrifugation and ultrafiltration from starved media after incubating SHSY5 cells and iPS-motor neuron cells for up to 48 hours. Exosome identity was confirmed by detecting known markers using fluorescence-activated cell sorting and western blot analysis. Co-immunoprecipitation via a GST-pulldown assay was used to identify potential binding partners of C9orf72.

Results: Quantitative analysis of the abundance of EV secretion by C9/ALS patient-derived fibroblasts and iPS-motor neurons, showed a marked reduction relative to healthy controls, following incubation in starved media from 4 to 16 hours, suggesting endosomal pathway dysfunction. Rab29, a member of a family of small cytosolic GTPases, has been previously implicated in intra-neuronal protein sorting. C9orf72 is predicted to contain a DENN-like domain, suggesting it has GTP-GDP exchange factor activity. Here we show Rab29 interaction with C9orf72 through co-immunoprecipitation. Also, transient expression of constitutive active/negative mutants of Rab29 with a flag-tag in C9/ALS patient-derived fibroblasts, revealed that C9orf72 physically interacts with the GTP-binding active form. This suggests C9orf72 may be an effector of Rab29. Furthermore, short interfering RNA knockdown of either Rab29 or C9orf72 in SHSY5 cells significantly reduced EV secretion, mimicking the changes seen in patient cells. Finally, gapmer-antisense oligonucleotide knockdown of mutant C9orf72 transcripts in C9/ALS patient-derived fibroblasts resulted in considerable rescue of the reduced EV secretion phenotype.

Conclusions: Assessment of the activity between Rab29 and C9orf72, suggests the latter may be a cofactor in intra-neuronal sorting, highlighting endosomal dysregulation as an important defect in C9/ALS. Also, reduction of EV secretion following C9orf72 knockdown suggests haploinsufficiency as a contributor of C9/ALS pathology. Finally, rescue of a disease-relevant phenotype by gapmer-antisense oligonucleotide suggest these may be a potential therapeutic strategy for C9/ALS.

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P193 MUTATIONS IN HNRNPA2/B1 AND HNRNPA1 LINKED TO MULTISYSTEM PROTEINOPATHY INDUCE ER STRESS AND ER-GOLGI TRAFFICKING DEFECTS REMINISCENT OF ALS PATHOLOGY

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Keywords: hnRNP, ER stress, trafficking

Background: Proteins implicated in ALS are functionally diverse with many linked to a spectrum of neurodegenerative diseases. Heterogeneous nuclear ribonucleoproteins A2/B1 (hnRNPA2/B1) and A1 (hnRNPA1) are highly homologous RNA processing proteins with major roles in mRNA metabolism. Mutations in both proteins were discovered in families with ALS and multisystem proteinopathy (MSP) (1). MSP is an inherited degenerative disease primarily affecting muscle, bone, brain and motor neurons, with reports of patients presenting with one or more indistinguishable features characteristic of ALS. This suggests common disease mechanisms may be shared between both diseases. The involvement of endoplasmic reticulum (ER) stress in the early stages of ALS has been well documented and intracellular trafficking is becoming increasingly implicated in disease pathogenesis (2, 3, 4). Elucidating disease mechanisms associated with hnRNPA2/B1 and A1 mutations may provide further insight into understanding both ALS and MSP.

Objectives: To determine whether MSP mutants hnRNPA2/B1^{D290V} and A1^{D262V} induce ER stress and/or cause ER-Golgi trafficking defects.

Methods: FLAG tagged wild-type (WT) and MSP mutants hnRNPA2/B1 and A1 were expressed with Venus tagged x-box binding protein 1 (XBP1), EGFP tagged C/EBP homologous protein (CHOP) or the mCherry tagged temperature sensitive mutant vesicular stomatitis virus glycoprotein (VSVG-ts045) in the human SH-SY5Y motor neuron like neuroblastoma cell line. ER stress and ER-Golgi trafficking were examined by immunofluorescence and immunocytochemistry.

Results: ER stress was quantified by nuclear XBP-1 and CHOP immunoreactivity (N=3). Nuclear XBP-1 and CHOP immunoreactivity was significantly higher in cells expressing either mutant compared to their respective WT counterparts. VSVG-ts045 misfolds in the ER at 40°C and refolds at 32°C then is transported to the Golgi. VSVG distribution was monitored by Calnexin (ER marker) and GM130 (Golgi marker) and quantified using the Mander's coefficient (N=3). At 32°C, VSVG-ts045 was transported to the Golgi in cells expressing WT proteins. Both mutants significantly inhibited transport of VSVG-ts045 to the Golgi however, cells expressing mutant hnRNPA2/B1 inibition was significantly greater. In addition mutant hnRNPA2/B1 also caused Golgi fragmentation.

Discussion and conclusions: We demonstrate MSP related mutations in hnRNPA2/B1 and A1 cause ER stress, ER-Golgi trafficking defects and golgi fragmentation reminiscent of ALS pathology, and provide further evidence that these disease mechanisms may be common among ALS and possibly MSP.

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P194 DEFECTS IN OPTINEURIN AND MYOSIN VI MEDIATED INTRACELLULAR AND AXONAL TRAFFICKING IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: optineurin autophagy axonal trafficking

Background: Mutations in OPTN gene have been identified to cause familial forms of Amyotrophic lateral sclerosis (ALS) (1) and Frontotemporal lobar degeneration (FTLD) (2). Wild-type (WT) optineurin is also misfolded and forms inclusions in sporadic ALS patient motor neurons (3). However, it is unknown whether or how loss of optineurin function, due to mutations or misfolding, could lead to neurodegeneration. Optineurin acts an adaptor protein connecting the molecular motor myosin VI to secretory vesicles and autophagosomes (4). The impact of optineurin ALS mutations or misfolding in cellular trafficking pathways remains unknown.

Methods: Immuoncytochemistry, immunoprecipitation and live confocal imaging were performed in: NSC-34 cells over-expressing WT and mutant optineurin constructs; human sporadic ALS patient spinal cord tissues; primary mouse cortical neurons; and zebrafish models expressing optineurin in motor neurons driven by the Hb9 promoter.

Results: In this study, we demonstrate that ALS-linked mutations p.Q398X and p.E478G disrupt the association of optineurin with myosin VI, leading to an abnormal diffuse cytoplasmic distribution, inhibition of secretory protein trafficking, endoplasmic reticulum (ER) stress and Golgi fragmentation in motor neuron-like NSC-34 cells. We also show that WT optineurin associate with lysosomes and promotes autophagosome fusion to lysosomes in neuronal cells, implying that it mediates trafficking of lysosomes during autophagy in association with myosin VI. However, either expression of ALS mutant optineurin or small interfering RNA-mediated knockdown of endogenous optineurin, blocked lysosome fusion to autophagosomes, resulting in autophagosome accumulation. In addition, in control human patient tissues, optineurin displayed its normal vesicular localization, but in sporadic ALS patient tissues, vesicles were present in a significantly decreased proportion of motor neurons. Optineurin binding to myosin VI was also decreased in tissue lysates from sporadic ALS spinal cords. Furthermore, in our recent studies using primary mice cortical neurons and zebrafish models, we have identified a novel role of the optineurin-myosin VI complex in vesicular trafficking of autophagosomes and lysosomes, along neuronal axons. This function is impeded by expression of myosin VI-binding deficient ALS-mutant optineurin, implicating defects in axonal trafficking, due to loss of optineurin function, in ALS.

Discussion and conclusions: This study therefore links several previously described pathological mechanisms in ALS, including defects in autophagy, fragmentation of the Golgi, induction of ER stress and axonal transport defects, to the disruption of optineurin function. These findings also indicate that optineurin-myosin VI dysfunction is a common feature of both sporadic and familial ALS.

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P195 TLQP PEPTIDES IN AMYOTROPHIC LATERAL SCLEROSIS (ALS): ANIMAL MODEL AND HUMAN STUDIES

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Keywords: VGF, neuronal apoptosis, trophic effect

Background: The neuronal VGF precursor yields a number of cleaved peptides, among these the TLQP peptides act on synaptic strengthening, or against neuronal apoptosis. The reduction of 4.8KD VGF fragments in cerebrospinal fluid (CSF) from ALS/MND patients has revealed the involvement of VGF in ALS/MND disease. Moreover in $SOD1^{G93A}$ transgenic mice, one of the most diffuse ALS/MND models, reduction of the total full-length VGF content in CSF and serum precedes the onset of ALS-type muscle weakness.

Methods: We assessed TLQP peptide profiles in $SOD1^{G93A}$ mice (n=10: pre-symptomatic; n=50: early and advanced stages) vs. wild-type (WT), (n=10), as well as in plasma from ALS/MND patients vs. age matched controls (n=50; 45-70 yrs) we used the motor neuronal NSC-34 cell line to identify possible roles for TLQP in neuronal survival after stress by Na-arsenite treatment (SA). We produced TLQP antisera to be used for immunohistochemistry and ELISA also coupled with gel chromatography.

Results: In lumbar and cervical motoneurons, TLQP immunoreactivity as identified by the vesicular acetylcholine transporter antibody, was mostly present in a number of perikaria of the WT mice, with a distinct decrease in the advanced stage of mutant SOD1 mice. In mouse plasma, TLQP immunoreactivity showed significant reduction in mutant SOD1 mice (20% of WT mice, $p<0.05$).

In the NSC-34 cell line, TLQP peptides were found in the growth cones. After SA treatment TLQP peptides were present in a perinuclear area and were found to be significantly decreased by ELISA. Furthermore, upon treatment with the VGF peptide TLQP-21 (1nmol/ml), the SA stressed NSC-34 neuronal cells seemed to show increased viability (113%). In ALS/MND patients TLQP immunoreactivity was significantly reduced (12% of controls, $p<0.05$) with the same decrease found in mouse plasma. ELISA coupled with gel chromatography showed that TLQP antiserum recognised a form complementary to the TLQP-21.

Conclusion: TLQP peptides appear to be involved in the ALS/MND neurodegenerative mechanisms in human, as well as in a mouse model, with a possibly trophic effect, which needs future investigation.

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P196 THE NOVEL ALS2-INTERACTING SMALL G PROTEIN RAB17 COLOCALIZES WITH ALS2 IN RECYCLING ENDOSOMES

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Keywords: ALS2/Alsin, Rab17, recycling endosome

Background: Amyotrophic lateral sclerosis 2 is an autosomal recessive form of ALS and is caused by loss-of-function mutations in the *ALS2* gene. ALS2/Alsin (the VPS9 domain-containing protein encoded by this gene), acts as a guanine nucleotide exchange factor (GEF) for small G protein Rab5 and regulates the fusion and maturation of early endosomes in cells. Recently, another small G protein Rab17 has been identified as a novel ALS2 interactor. Rab17 is known to be localized to dendrites and soma but not to axons in mouse hippocampal neurons, and is activated by RabGEF1 (another VPS9 family protein) thereby regulating dendritic morphogenesis. However, the functional relationship between ALS2 and Rab17 remains to be studied.

Objectives: To gain insight into the ALS2 functions that are associated with Rab17, we investigated the subcellular localization of ALS2 and Rab17 in HeLa cells.

Methods: We transfected expression plasmids encoding ALS2 or Rab17 into HeLa cells and stained the target molecules by immunocytochemistry. Immunofluorescent signals were detected by confocal laser scanning microscopy.

Results: We first investigated the localization of Rab17 (endogenous Rab17, and ectopically-expressed $Rab17^{WT}$, $Rab17^{CA}$, and $Rab17^{CN}$). Both endogenous Rab17 and $Rab17^{WT}$ were mainly localized to the perinuclear compartment. A subpopulation of $Rab17^{WT}$ was distributed to tubular structures and enlarged vesicles. On the other hand, $Rab17^{CA}$ and $Rab17^{CN}$ signals were predominantly detected in the cytoplasm. Next, we examined the colocalization of ALS2 and Rab17. The majority of ALS2 and $Rab17^{WT}$ were colocalized in the perinuclear compartment. ALS2 was also detected in $Rab17^{WT}$-positive tubular structures and enlarged vesicles. Moreover, overlapping distribution of ALS2 and $Rab17^{WT}$ at the tip of filopodia was observed. Since the $Rab17^{WT}$-positve structures resemble not only those for early endosomes but also for recycling endosomes, we tested whether Rab5, an early endosome marker, or Rab11, a recycling endosome marker, was present in the $Rab17^{WT}$-positive structures. Both $Rab17^{WT}$ and $Rab17^{CA}$, but not $Rab17^{CN}$, were extensively colocalized with Rab11, while a subpopulation of $Rab17^{WT}$ was present in Rab5-positive early endosomes. Interestingly, $Rab17^{WT}$-positive tubular structures resided along microtubules.

Discussion and conclusions: These results suggest that ALS2 plays some roles not only in the regulation of early endosome fusion and trafficking via the activation of Rab5, but also in the trafficking of recycling endosomes along with Rab17 and Rab11. Further studies on the functional relationships between ALS2 and Rab17 may provide additional clues to understand the molecular mechanisms underlying the pathogenesis of recessive motor neuron diseases caused by loss-of-function mutations in the ALS2 gene.

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P197 ALS ASSOCIATED MUTATIONS IN MATRIN 3 ALTER PROTEIN BINDING PARTNERS

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Keywords: Matrin 3, proteomics, protein interactions

Background: We recently described four mutations in the *Matr3* gene encoding the nuclear matrix protein Matrin 3 that are associated with ALS. We have shown using human spinal cord sections that Matrin 3 immunostaining is predominantly nuclear and appears more intense in ALS patients than in control cases, with the strongest nuclear immunoreactivity occuring in an ALS patient with a *Matr3* mutation (1). Matrin 3 does not appear to exhibit gross mislocalization or the widespread formation of inclusions in ALS tissue as is often seen in other proteins involved in ALS. Therefore we have chosen to explore its role and protein interactions within the nucleus.

Objectives: To determine novel binding partners of Matrin 3 and to determine which protein interactions are altered by ALS causing mutations.

Methods: Cell lines stably expressing each of the four mutations and wild type Matrin 3 have been created in HEK cells and NSC-34 motor neuron like cells. Immunoprecipitation experiments were performed in these cell lines followed by separation by gel electrophoresis and trypsin digestion. Peptides were extracted and analyzed on a Thermo LTQ Orbitrap Velos mass spectrometer. Interactions were validated with immunoprecipitation followed by western blot. Proteins that bind to wild-type Matrin 3 were compared to proteins binding each Matrin 3 mutant, yielding a list of protein interactions altered by ALS linked mutations.

Results: The top biological processes of proteins shown to bind Matrin 3 include rRNA processing; RNA/DNA helicases; chromatin assembly; RNA binding proteins; histone core proteins; nuclear division; nucleotide binding; RNA splicing; and transcription factors. The interaction between Matrin 3 and TDP-43 was again confirmed by these experiments. We also confirmed Matrin 3 protein interactions previously reported by others, including SAFA (3), hnRNPL (2,3) and PTBP1 (2). Novel protein interactions between Matrin 3 and proteins involved in ALS pathogenesis or other neurodegenerative diseases were also found.

Discussion and conclusions: Mutations in Matrin 3 are novel to the field of ALS and neurodegeneration. Our

current study provides novel insight into the numerous functional roles of Matrin 3 within the nucleus and the many cellular processes that rely on Matrin 3. This work identifies cellular processes attributed to Matrin 3 that are disrupted in ALS. Moreover we have discovered links between Matrin 3 and proteins implicated in familial forms of ALS and other neurodegenerative diseases.

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P198 DISSECTING THE INTERACTOME OF RBM45 USING IMMUNOPRECIPITATION AND MASS SPECTROMETRY

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Keywords: RNA-binding protein, RBM45, mass spectrometry

Background: A proteomic analysis of cerebrospinal fluid (CSF) from sporadic ALS and healthy control subjects identified increased levels of RBM45 in the CSF of sporadic ALS patients (1). RBM45 containing cytoplasmic inclusions that co-localized with TDP-43 and ubiquitin were also observed in affected neurons in both ALS and FTLD patients (1). Recently, a role of RBM45 in the antioxidant response in ALS was reported (2). However, little is known about the protein interactome of RBM45 and its normal function.

Objective: Identify biochemical pathways in which RBM45 functions by determining the protein-protein interactome of RBM45.

Methods: The protein interactome of RBM45 was studied via immunoprecipitation-mass spectrometry (IP-MS). Different epitope-tagged RBM45 were overexpressed in HEK293 cells and immunoprecipitated. The co-immunoprecipitated proteins were identified by liquid chromatography tandem mass spectroscopy (LC-MS/MS). Top hits were generated by the list of intersecting proteins in a Venn diagram using results from different epitope tagged RBM45 constructs, eliminating any proteins identified using control IPs. Regular IP-MS and formaldehyde crosslinking IP-MS were performed in parallel to identify the strongly bound proteins, as well as the weakly bound proteins.

Results: We identified 130 proteins that specifically interact with RBM45 with high confidence using IP-MS. The top hits were validated by IP-western blot and co-localization experiments in cultured cells. Gene ontology analysis suggests that RBM45-interacting proteins are predominately enriched in two clusters: the nuclear RNA processing cluster and the cytoplasmic translation cluster. Our IP-MS results also show that RBM45 interacts with a number of ALS-linked proteins, including TDP-43, FUS, Matrin 3, hnRNP-A1, and hnRNP-A2/B1.

Discussion: We characterized RBM45-interacting proteins and further defined the functional roles of wild-type RBM45. The gene ontology analysis results are consistent with RBM45s role as both a RNA-binding moiety and protein-binding moiety, which can shuttle between the cytoplasm and nucleus to perform distinct cellular functions. The protein-protein interactions between RBM45 and other ALS-linked proteins may modulate its functions during disease and warrants further investigation.

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P199 THE IMPACT OF RHO KINASE (ROCK) AND PHOSPHATASE AND TENSIN HOMOLOGUE (PTEN) CROSS-TALK ON THE NEURONAL CYTOSKELETON IN AMYOTROPHIC LATERAL SCLEROSIS AND SPINAL MUSCULAR ATROPHY

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Keywords: cytoskeleton, FUS, Rho-kinase

Background: Depletion of the Survival of Motoneuron (SMN) protein leads to the motor neuron disease Spinal Muscular Atrophy (SMA). The amyotrophic lateral sclerosis (ALS) associated and mutated gene product Fused in sarcoma (FUS) directly binds to SMN and sequesters the protein in the cytoplasm. Therefore, both diseases putatively share common pathomechanisms linked by SMN. SMN is an important assembly factor for small nuclear ribonucleoprotein particles (snRNPs) involved in pre-mRNA splicing. It was shown that these snRNPs are

diminished in both diseases. Moreover, we previously reported that SMN is also involved in the regulation of the actin cytoskeleton via Rho-kinase (ROCK) (1,2). In addition, it has been shown that inhibition of ROCK ameliorates disease progression in cell culture and mouse models of SMA and ALS. ROCK interacts with the phosphatase and tensin homologue (PTEN) and inhibition of PTEN is also protective in SMA and ALS by upregulating the anti-apoptotic kinase Akt. As ROCK directly regulates PTEN activity, we hypothesise that PTEN also impacts on the neuronal cytoskeleton.

Objectives: To assess (1) the functional binding site of SMN on FUS and (2) the influence of PTEN inhibition on the neuronal cytoskeleton in ALS and SMA.

Methods: For identification of FUS-binding sites on SMN, a cDNA-library coding for full-length or mutant SMN was used and co-immunoprecipitations were performed (3). To analyse the impact of PTEN on the neuronal cytoskeleton, a PTEN inhibitor was used in models of ALS and SMA to unravel the induced signal transduction on the actin cytoskeleton.

Results: FUS mutations increased SMN binding and altered localisation of both proteins. Moreover, we were able to identify the binding site of SMN on FUS. To analyse the impact of PTEN inhibition on the cytoskeleton, cells were treated with siRNA or scrambled RNA against SMN and differentiated for 72 hours. We found that the ROCK downstream target cofilin is hypophosphorylated in siRNA-treated cells compared to control. When the cells were incubated with a PTEN inhibitor, cofilin phosphorylation was increased in knockdown cells whereas the phosphorylation did not differ in vehicle- or inhibitor-treated control cells. On the other hand, we and others showed that PTEN inhibition led to increased Akt phosphorylation in both conditions.

Discussion and conclusion: SMN function is disturbed in both, SMA and ALS. These results suggest that SMN function is not crucial for PTEN function in general but for ROCK-PTEN cross-talk. Thus, PTEN inhibition is not only beneficial by upregulating anti-apoptotic signals but also by restoring disturbed cytoskeletal regulation.

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Theme 11 ALS Pathogenesis and Neurotoxicity

P200 UNRAVELING COMMON MECHANISMS BETWEEN MANGANESE NEUROTOXICITY AND ALS-RELATED MOTOR NEURON DEATH

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Keywords: manganese, astrocytes, amyloid precursor-like protein 1

Background: Manganese (Mn) is a trace element vital for human health as it serves as a cofactor for many enzymes critical for development, metabolism and redox homeostasis. However, excessive exposure to Mn results in a severe neurological disorder referred to as "manganism". Its clinical expression is classically described to mainly resemble Parkinson's disease, but at lower levels, chronic Mn overexposure has more subtle effects on cognitive and motor abilities, some reminiscent of ALS, such as muscle weakness. Mn smelters and miners have been described to develop both ALS and manganism in different parts of the world. Mn overload was also suggested to be implicated in the high incidence of the ALS-Parkinson's Dementia Complex in Guam and the Kii Peninsula. Finally, increased Mn levels were measured in diverse bodily fluids and in the spinal cord of ALS patients and animal models.

Previously, we showed that motor neuron degeneration in SOD1-linked ALS may be initiated by neighboring astrocytes which undergo a transformation from supportive cells to neuron killers. Recently, we have developed an *in vitro* model of human sporadic ALS by co-culturing astrocytes produced from sporadic ALS patients and embryonic stem cell-derived motor neurons. Interestingly, we found that the expression of a protein called amyloid precursor-like protein 1 (APLP1) is increased in ALS astrocytes and may be responsible for their toxic effect to motor neurons. Independently, our collaborator, Dr. Guilarte, has reported increased APLP1 levels in the brain of macaques chronically treated with Mn.

Objectives: 1) to investigate the neurotoxicology of Mn in the spinal cord of chronically-exposed macaques; 2) to determine whether increased APLP1 levels are responsible for neuronal death upon Mn exposure.

Results: Preliminary histological investigation indicates the presence of shrunken and chromatolytic motor neurons in the ventral horn of the lumbar spinal cord of macaques exposed to Mn for 14 months, as compared with vehicle-injected animals. *In vitro* we have shown that Mn is dose-dependently toxic to motor neuron soma and processes at levels equivalent to those reached in the brain of exposed macaques (2.5 μM). We have also found that APLP1 expression is progressively and dose-dependently increasing in astrocytes and astrocyte/neuron co-cultures over 4 weeks of Mn treatment. We are currently confirming whether Mn toxicity to motor neurons exhibits both a cell-autonomous and non-cell autonomous component and its dependence on APLP1.

Discussion and conclusions: Altogether this study suggests that Mn is neurotoxic to spinal motor neurons both *in vivo* and *in vitro*. It may unravel a novel molecular link between neurodegenerative conditions such as ALS and manganism, and identify new targets for both the prevention and therapy of neurodegeneration.

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P201 BMAA ENANTIOMERS IN THE CENTRAL NERVOUS SYSTEM: IMPLICATIONS FOR NEURODEGENERATIVE DISEASE

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Keywords: Guam, ALS/PDC, BMAA

Background: As the majority of sporadic ALS cases have no known cause, environmental factors are believed to play a role in gene/environment interactions in the etiology of neurodegenerative disease. Cyanobacteria are commonly-occurring photosynthetic bacteria which produce small molecular weight compounds of high toxicity. One such cyanobacterial toxin, BMAA (1), has been linked to Guam ALS/PDC (2).

Objectives: As the L-enantiomer of BMAA is the only one that has previously been detected in nature, we sought to determine if the D-enantiomer occurs in the natural environment and whether processes in the central nervous system of mice and vervets fed with L-BMAA could convert this neurotoxic amino acid into the D-enantiomer.

Methods: Mice and vervets were exposed to chronic dietary L-BMAA. Samples of blood serum and CSF (vervets) and liver and hindbrain (mice) were subsequently collected and analyzed for the presence of BMAA by triple quadrupole LC-MS/MS. Samples that were positive for BMAA underwent chiral HPLC separation

and fractionation for BMAA enantiomers and isomers, and the fractions subsequently assessed by LC-MS/MS.

Results: Samples of animals with chronic dietary exposure to L-BMAA were found to contain BMAA. Chiral analysis of free BMAA indicated that serum and liver samples were predominantly L-BMAA, suggesting that enantiomeric changes do not occur during digestion. However in the central nervous system only the D-enantiomer of BMAA was detected.

Discussion and conclusions: Previous *in vitro* and *in vivo* studies have focused on the neurotoxicity of L-BMAA, so little research has considered the possibility of the existence of D-enantiomers of neurotoxic and excitotoxic amino acids (3). We find that enantiomeric changes to dietary L-BMAA do not occur during digestion, but, surprisingly, in the central nervous system only the D-enantiomer of BMAA occurs as a free amino acid. This suggests that a mechanism exists in the central nervous system to change the chirality of L-BMAA. As a result, the enantiomeric conversion of BMAA in the central nervous system could produce a different toxicological outcome to that indicated by *in vitro* studies of L-BMAA. Further research is required to understand the presence and toxicity of D-BMAA in the central nervous system and its association with human neurodegenerative disease.

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P202 DIRECT BINDING OF B-N-METHYLAMINO-L-ALANINE TO PROTEINS CONTRIBUTES TO ITS TOXICITY

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Keywords: BMAA, toxicity, protein-misfolding

Background: β-N-methylamino-L-alanine (BMAA) produces symptoms corresponding to neurodegeneration in animal models[1–3] and neurotoxicity in neuronal cultures[4–6]. Published mechanisms of toxicity are excitotoxicity of the β-carbamate adduct[7], misincorporation into proteins and protein misfolding[6], and inhibition of cystine uptake through the cystine/glutamate antiporter[8] resulting in oxidative stress.

Objectives: We sought to discover novel independent or contributory mechanisms of BMAA toxicity by demonstrating *in vitro* protein-BMAA binding with consequent effects on protein folding in the absence of misincorporation, and on enzyme activity.

Methods: BMAA-protein interaction was investigated by *in vitro* co-incubation of BMAA with selected proteins and subsequent treatment of the proteins with denaturants such as SDS and DTT, and combinations of these. BMAA content of proteins was determined by mass spectrometry[9]. Protein misfolding was investigated by dilution refolding of urea-denatured RNase with standard activity assays, and by thermal refolding of TDP 43 in the presence of SYPRO orange. Various enzymes including catalase, peroxidase, glutathione-reductase and superoxide-dismutase were investigated for BMAA inhibition using standard analytical methods .

Results: *In vitro* binding studies confirmed BMAA-protein binding in cell free systems. BMAA could not be removed by DTT and/or SDS but could be removed by hydrolysis. *In vitro* protein folding in the presence of BMAA resulted in a loss of function of RNase A. Dose dependent inhibition of relevant oxidative stress response enzymes was observed *in vitro* and *in vivo*.

Discussion and conclusions: The disruption of protein structure and function by direct, strong binding of BMAA constitutes a mechanism of BMAA toxicity in the absence of glutamate or AMPA/kainate receptors and in the absence of translation. We therefore conclude that the direct interaction of BMAA with proteins may exacerbate the toxicity of BMAA by reducing the cell's ability to respond to or counter excitotoxic or ER stress caused by BMAA, and may constitute a novel mechanism of toxicity.

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P203 PROLONGED INTRATHECAL INFUSION OF BETA-N-METHYLAMINO-L-ALANINE (BMAA) INDUCES PREFERENTIAL MOTOR NEURON DAMAGE AND ASTROGLIOSIS IN THE VENTRAL HORN OF THE SPINAL CORD

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Keywords: BMAA, intrathecal, animal model

Background: The neurotoxin beta-methylamino-L-alanine (BMAA) was first identified as a "toxin of interest" in regard to the amyotrophic lateral sclerosis-Parkinsonism Dementia Complex of Guam (ALS/PDC). Recent studies highlighting widespread environmental sources of BMAA exposure and providing new clues to toxic mechanisms have suggested possible relevance to sporadic ALS as well.

Objectives: Despite clear evidence of uptake into tissues and a range of toxic effects in cells and animals, an animal model in which BMAA induces a neurodegenerative picture resembling ALS is lacking. In order to bypass incompletely characterized factors related to absorption, biodistribution and metabolism, we opted to ensure delivery to a key site of disease pathology, by carrying out prolonged intrathecal infusions in wild type (WT) and G93A SOD1 mutant rats (1).

Methods: A catheter was inserted into the subarachnoid space of anestensized rats advanced to the lumbar enlargement, and connected to an Alzet mini-osmotic pump which was pre-filled with 5 mM BMAA or saline. Infusions were carried from 80±2 to 110±2 days of age, a period during which the SOD1 mutant rats develop disease pathology yet remain asymptomatic.

Results: BMAA exposures induced changes resembling those seen in the G93A rats: damage to ventral horn motor neurons (MNs) with relatively little dorsal horn pathology; marked ventral horn astrogliosis; increased 3-nitrotyrosine labeling in and surrounding MNs; a loss of labeling for the astrocytic glutamate transporter, GLT-1, surrounding MNs; mild accumulation and aggregation of TDP-43 in the cytosol of some injured and degenerating MNs. Notably, while the BMAA infusion and the SOD1 mutation each resulted in marked pathological changes, under the exposure conditions employed, these mechanisms showed little synergism, as changes in BMAA infused G93A rats were little greater than those resulting from BMAA or mutant SOD1 alone.

Discussion and conclusions: In prior studies we found BMAA to be an unusual excitotoxin, that activated glutamate receptors only in the presence of bicarbonate ions. Also, while it was a weak NMDA agonist, it induced selective injury to subpopulations of neurons including MNs via AMPA receptor activation. More recent studies suggest other mechanisms contributing to its neurotoxicity, including possible misincorporation in peptide chains. While its toxic mechanisms are incompletely understood, present findings indicate that protracted intrathecal infusion of BMAA can reproduce a picture in spinal cord incorporating many of the pathological hallmarks of diverse forms of human ALS, including substantial restriction of overt pathological changes to the ventral horn, consistent with the possibility that environmental BMAA exposure could be a risk factor or contributor to human disease.

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P204 UPTAKE AND PROTEIN-ASSOCIATION OF THE ENVIRONMENTAL NEUROTOXIN BMAA (B-N-METHYLAMINO-L-ALANINE) IN THE NEONATAL CENTRAL NERVOUS SYSTEM

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Keywords: neurotoxin hippocampus neonatal

The environmental toxin β-methylamino-*L*-alanine (BMAA) is implicated in the etiology of neurodegenerative disorders and the mechanism of neurotoxicity is of concern. BMAA is a developmental neurotoxin that induces long-term cognitive deficits and progressive neurodegenerative changes with intracellular fibril formation in the hippocampus of adult rats following neonatal exposure. Enrichment of proteins implicated in protein aggregation, and an increased protein ubiquitination, are observed in the adult hippocampus following neonatal exposure. This indicates that the non-protein amino acid BMAA could contribute to protein misfolding and/or protein aggregation that are hallmarks of many neurodegenerative disorders. Ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) analysis revealed that free BMAA was present in relatively high concentrations in the neonatal brain compared to peripheral tissues, except the liver, demonstrating that BMAA is readily passing the neonatal blood-brain barrier. BMAA was also associated to proteins in the neonatal brain, especially in the hippocampus. However, the level of protein association in the hippocampus was considerably lower than that of the liver, which is not a target organ for BMAA in neonatal rodents. The protein-association of BMAA in the hippocampus and other brain areas increased following repeated administration of BMAA in neonates, whereas the hepatic level of protein-associated BMAA did not increase, suggesting that the degradation of BMAA-associated proteins may be different in the neonatal brain and liver. Additional evidence is needed in support of a role for protein misincorporation of BMAA in the neonatal hippocampus for the selective toxicity of BMAA in this brain region.

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P205 LOCOMOTOR AND ELECTROPHYSIOLOGICAL EFFECTS OF AN L-BETA-METHYLAMINO-ALANINE (L-BMAA) FED DROSOPHILA MELANOGASTER MODEL OF ALS-PDC

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Keywords: BMAA, Drosophila melanogaster, electrophysiology

Background: Amyotrophic lateral sclerosis-Parkinsonism dementia complex (ALS-PDC) is a triad of neurodegenerative diseases characterized by loss of motor function, tremors, and dementia. L-BMAA (L-beta-methyl amino alanine), a non-natural amino acid, is implicated in the multiple etiologies of ALS-PDC. Exposure to BMAA may be biomagnified from many species of cyanobacteria that produce BMAA, to the symbiotic root nodules of the cycad plants or to other sources within the food chain. It is now known that BMAA is present worldwide and may be implicated in cases of ALS.

Objectives: The mechanism of BMAA and how it leads to or causes ALS-PDC is not clear, however, animal models play an important role in the elucidation of this mechanism. The goal of our research is to test if *Drosophila melanogaster* (fruit fly) is a good model for studying ALS and the mechanisms related to locomotion and neurotransmission.

Methods: Canton S (wildtype) flies were fed BMAA at varying concentrations (12.5, 25, and 50 mM) to model the ingestion route of exposure similar to that of humans. Climbing performance was tested using the simple tap-down test and more complex locomotion and activity was measured using a lenticular arena to measure daily activity. Behavior such as activity levels were also measured; all locomotor and electrophysiology experiments were conducted over a 3-day feeding period. Post-synaptic responses of the dorsal longitudinal flight muscle (DLM) fibers were measured intracellularly using standard electrophysiological techniques.

Results: The BMAA-fed flies (n=10) showed a decreased ability to climb (60 mm wall in 10 secs) in a time and dosage dependent manner. Climbing performance (% of total flies; n = 5-6 trials) indicated that the BMAA-fed flies began losing locomotor ability even after one day of treatment: 12.5 mM (78.9 ± 7.8); 25 mM (66.5 ± 13.1); 50 mM (14.8 ± 8.9), whereas the untreated control (85.6 ± 7.0) flies were able to climb; $p<0.05$. There was also a dosage-dependent hyperactivity followed by eventual loss of motor control or death (1). The intracellular recordings of the DLM again showed a dose-dependence, with depolarization of NMDA receptor channels at the glutamatergic neurons (2).

Discussion and conclusions: The dosage dependent locomotor deficiencies, hyperactivity, and depolarization of the NMDA receptors in a fed-fruit fly model recapitulate similar symptomatic features of ALS-PDC. This demonstration of the fly model of ALS-PDC leads the way for characterizing and screening small molecules that may serve to reverse or decrease the effects of ingested BMAA, alone.

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P206 CHRONIC DIETARY EXPOSURE TO BMAA IN VERVETS RESULTS IN BMAA BRAIN CONCENTRATIONS SIMILAR TO GUAM ALS/PDC PATIENTS

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Keywords: Guam, ALS/PDC, BMAA

Background: The cyanobacterial toxin β-N-methylamino-L-alanine (BMAA) has been linked with Guam Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex (ALS/PDC) since it produces neuropathological changes in nonhuman primates (1, 2).

Objectives: We sought to 1) confirm if dietary exposures to BMAA result in BMAA crossing the blood brain barrier, and 2) quantify BMAA concentrations in brain, cerebral spinal fluid (CSF), and blood resulting from chronic dietary exposure to BMAA.

Methods: Vervets (*Chlorocebus sabaeus*) were fed fruit dosed with BMAA, BMAA plus L-serine, or rice flour for 140 days in a replicated experiment. Blood and CSF samples were collected throughout the study. BMAA was analyzed in blood, CSF, and brain tissues using triple quadrupole LC-MS/MS. Protein precipitation of tissues allowed for analysis of both free BMAA and protein-bound BMAA.

Results: BMAA was detected in the samples from cohorts fed BMAA, and BMAA plus L-serine, but was not detected in control cohorts or in any of the samples taken prior to BMAA feeding. In those vervets fed BMAA, final concentrations of free BMAA in CSF (0.2 - 5 μg/ml, mean 1 μg/ml) were lower than in blood plasma (4 - 126 μg/ml, mean 27 μg/ml), similar to plasma:CSF ratios reported for canonical amino acids. Free BMAA was detected in postmortem brain tissues (3 - 51 μg/mg wet wt, mean 19 μg/ml), confirming BMAA transport across the blood-brain barrier. Protein-bound BMAA was measured in brain tissues at concentrations ranging from 0.3 - 2 μg/mg, similar to those previously quantified in

Chamorro ALS/PDC brain tissues (0.15 - 1.2 μg/mg) (3,4). Mean protein bound BMAA was 0.9 μg/mg in brain tissues and 3 μg/ml in plasma. Animals fed BMAA and L-serine had half the free/protein bound BMAA ratio in their blood plasma as compared with animals receiving only BMAA (7 to 14 respectively).

Discussion and conclusions: BMAA is present in the blood, CSF, and brain of vervets with chronic dietary exposure to BMAA; these same animals developed protein inclusions. These data support the suggestion that chronic dietary exposure to BMAA in individuals who later developed ALS/PDC accounts for BMAA measured in their brain tissues (4). Quantitative correlations between BMAA concentrations in brain tissue and neuropathology are needed to better understand relevant BMAA concentrations and disease etiology.

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P207 BMAA INDUCES PROTEOTOXIC STRESS IN A NOVEL *IN VITRO* ALS MODEL

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Keywords: BMAA, vervets, ER stress

Background: BMAA, a non-protein amino acid produced by cyanobacteria, has been linked to Guam ALS/PDC and elsewhere to sporadic ALS. BMAA functions as an excitotoxin at glutmatate receptors, killing subpopulations of NADPH-diaphorase-positive motor neurons through multiple mechanisms (1, 2). BMAA can be misincorporated for L- serine, causing protein misfolding and cell death (3). ER stress has been implicated in BMAA-induced neuronal cell death *in vitro* (4). We sought to determine if similar intracellular stress responses to BMAA are present in a novel non-human primate model.

Objectives: To determine if proteotoxic stress is induced in non-human primates with chronic dietary exposure to BMAA.

Methods: Brain tissues from vervets (*Chlorocebus sabaeus)* with chronic dietary exposure to BMAA were compared to brain tissues from control vervets. RNA and protein were extracted using Sigma TRI Reagent, cDNA was synthesised with BioRad iScript, custom primers were designed and optimised in accordance with MIQE guidelines and samples analysed by qPCR. A total of 2 μg total protein was used for western blotting. Proteins were visualised with 3,3′-diaminobenzidine-tetrahydrochloride and images acquired/quantitated using a Canon 5D Mark III and Image J.

Results: We report a significant increase in the expression of spliced x-box binding protein (sXBP1) gene ($p < 0.05$), a UPR transcription factor involved in ER stress, in BMAA-fed vervets versus controls. We also identified significant upregulation of PP2A ($p < 0.05$), a protein implicated in the development of tauopathies. mRNA levels for PP2A were unchanged, suggesting the pathway by which BMAA triggers tauopathy is post-translational.

Discussion and conclusions: Proteotoxic stress has been implicated in ALS and in cell cultures and rats treated with BMAA (3-5). Here, we report that ER stress also occurs in non-human primates chronically exposed to dietary BMAA. These results suggest that proteotoxic stress could be involved in BMAA-induced neurodegeneration.

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P208 CYANOBACTERIAL TOXINS AND THEIR ISOMERS IN DESERT SOILS

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Keywords: cyanotoxins, desert soils, BMAA

Background: Desert regions and arid soils are stabilized by a crust of cyanobacteria that bind sand. Cyanobacteria are known to produce a variety of toxins (1,2,3), including those associated with acute and chronic neurological responses. Although desert cyanobacteria are dormant for most of the year, they can become metabolically active within minutes of receiving rainfall.

Objectives: In this study we tested whether cyanobacteria in desert biological soil crusts produce neurotoxins such as

diaminobutyric acid (DAB), N-(2-aminoethyl)glycine (AEG) and beta-N-methylamino-L-alanine (BMAA) which has been linked to ALS/MND. We also tested the hypothesis that toxin production in biological soil crusts varies depending on rainfall and disturbance regimes in arid environments.

Methods: Four quadrats in five different plots were watered, disturbed and ultimately monitored and tested for the presence of three cyanobacterially-produced toxic isomers BMAA, DAB, and AEG for a period of five days. Surface soil crusts were collected from each quadrat and three separate plots were sampled at depth. All samples were then hydrolyzed with hydrochloric acid, centrifuge filtered, rehydrated and analyzed with LC-MS/MS.

Results: We found that all three neurotoxic isomers, BMAA, DAB and AEG were present in the sampled plots and that the production of these isomers was not correlated with watering regime and increased metabolic activity ($P > 0.05$). When we looked at the toxins at depth (from 0-25 cm), DAB significantly decreased from 0-25 cm ($P=0.007$) and BMAA also significantly decreased with depth in the soil profile ($P=0.029$). In addition, AEG ($P=0.034$) and DAB ($P=0$) were significantly correlated with BMAA.

Discussion and conclusions: Our results show that low molecular weight cyanotoxins produced by biological soil crusts are persistent in the soil, and at depth, even in the absence of crust material. Thus, over many years a potentially large reservoir of cyanobacterial toxins can accumulate in desert soils, with possible health consequences for human exposure to particulate matter in dust storms, and from disturbance of desert soils from construction and military activities that result in loss of soil structure. Mechanical disturbance of soil either at the surface or at a profile depth of 25 cm in both dry and wet conditions may equally contribute to human exposure to cyanotoxins through inhalation (1).

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P209 THE INTERACTION OF THE ENVIRONMENTAL NEUROTOXIN BMAA (BETA-METHYLAMINO-L-ALANINE) WITH MUTANT SOD1 IN A ZEBRAFISH MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: genetic-environmental, BMAA, zebrafish

Background: The scientific consensus is that gene-environment interactions are key for the development and progression of ALS, but how either toxicants or genes lead to a disease mechanism is currently unknown. This represents a major gap in our understanding of the pathogenesis of ALS. A suite of environmental neurotoxicants has been associated with ALS, with evidence indicating that early developmental exposures to neurotoxins can have consequences for neurotoxicity later in life. Early defects in neural circuitry have also been found to be associated with late-onset neurological disorders, including both cognitive and degenerative diseases. Potentially, impairment of genes crucial to early neuronal differentiation makes neurons more susceptible to additional environmental disruptions. By determining cellular pathways involved in modifying neurological defects we hope to gain a better understanding of the root causes of this disorder.

Objectives: The zebrafish (*Danio rerio)*, has been shown to be a robust model organism for modeling human neurodegenerative diseases, including ALS. Our research aims to study the intersection of genetics and environmental neurotoxins on both developmental motor neuron defects and on adult-onset disease in a zebrafish model of ALS.

Methods: We have determined the impact of embryonic exposure to environmentally relevant doses (0-25μg/L) of the ALS-associated cyanobacterial neurotoxin β-methylamino-L-alanine on early neurological defects in mutant SOD1-ALS zebrafish (both transient mRNA-injected SOD1-G93A and transgenic SOD1-G93R fish), and on consequences for adult motor function in transgenic SOD1-G93R fish.

Results: SOD1-G93A mRNA-injected zebrafish exhibit significantly shorter 30hpf motor neurons at low doses (2.5μg/L BMAA), while control fish are impacted at intermediate doses and SOD1-wt overexpressing fish show a trend towards increasing nerve length at higher doses.

Tg-SOD1-G93R zebrafish exhibit significantly shorter 30hpf motor neurons at medium doses (10μg/L BMAA) but Tg-SOD1-WT overexpressing embryos are not impacted at all.

Five-month old Tg-SOD1-G93R fish (embryonically exposed to BMAA) show decreased ability to swim against water current, with increasing embryonic BMAA dose having a negative impact on swimming ability. In contrast, Tg-SOD1-WT fish exhibit an increased swimming ability with increasing BMAA dose.

Five month Tg-SOD1-G93R fish also show increasing fatigue when repeatedly challenged in the water current, while Tg-SOD1-WT fish do not exhibit any change in swimming over repeated challenges.

Discussion and conclusions: Our results indicate that genetic and environmental insults combine to facilitate neurological dysfunction in ALS, and that overexpression of wt-SOD1 may have protective effects against neurotoxin damage. The defects seen in early neurodevelopment are

mirrored at 5 months of age in the ability of fish to swim against a current and to fatigue with repeated swimming challenges. We are presently investigating older cohorts to determine the progression of this motor dysfunction, and to determine gene expression and proteomic changes in motor neurons as the fish age.

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P210 NEUROPATHOLOGIC EXAMINATION OF AFRICAN GREEN MONKEYS EXPOSED TO CYANOBACTERIAL TOXIN BMAA

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Keywords: BMAA, cyanotoxin, primate

Background: BMAA (β-N-methylamino-l-alanine) is a non-protein amino acid produced by cyanobacteria. Recently, BMAA has been measured in the brains of patients with Guam ALS/PDC and North Americans with Alzheimer's disease and ALS. Since BMAA is ubiquitous throughout the world, it is possible that many humans are exposed to low amounts of BMAA from their diets. The accumulation of protein-bound BMAA in human brain is a reservoir for slow neurotoxicity, which makes BMAA a potential environmental toxin in progressive neurodegenerative diseases. We used the vervet (African Green Monkey) to conduct a neuropathology assessment of low dose chronic dietary exposures to BMAA.

Methods: Adult African green monkeys (*Chlorocebus sabaeus*; 4.1–6.7 kg body weight) were collected and studied under the auspices of the St. Kitts Biomedical Research Foundation (St. Kitts, West Indies). Vervets were fed oral BMAA doses for 140 days and compared to control monkeys that received rice flour or L-serine. The brains of seventeen vervet monkeys were examined with thioflavin-S and compared to Aβ and tau immunohistochemistry (AT8, Thr205; Neuroscience Associates, Knoxville, TN).

Results: Although the vervet is known to be homozygous for the apoliprotein E4 allele, the brains of the young adult vervets did not contain senile plaques as assessed by anti-Aβ immunostains and no neurofibrillary tangles (NFTs) are visible using the AT8 antibody. Aβ staining of BMAA fed vervets revealed weak labeling of the neuropil and more intense staining in the frontal and entorhinal cortices. Aβ plaques were visualized in the entorhinal cortex and amygdala with scattered rare plaques seen in motor, temporal, and occipital cortices in BMAA fed monkeys. Astrocytes were labeled with the AT8 antibody and CNS tau inclusions appeared to be similar to ALS/PDC and Alzheimer's disease (AD) pathology. Intraneuronal aggregates in cortical neurons stained with AT8 resembled NFTs. Thioflavin-S fluorescent microscopy confirmed flame-like and spherical appearances of NFTs. More abundant NFTs and dystrophic neurites were visualized in aged vervets fed oral BMAA doses, suggesting an age-related increase in tau aggregation with chronic BMAA exposures.

Conclusions: The vervet was selected as a primate model for assessing neuropathology of chronic BMAA exposures. BMAA fed vervets demonstrate glial and neuronal protein inclusion pathology. Although the role of BMAA in human neurodegenerative disease is still highly debated, the results suggest a possible toxic role for BMAA that contributes to human disease.

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P211 PROTEOMIC CHARACTERIZATION OF CYTOPLASMIC FUS AGGREGATES

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Keywords: mass spectrometry, mRNA metabolism, protein aggregation

Background: Mutations in Fused in Sarcoma (FUS) contribute to roughly 5% of all fALS cases (1). FUS contains several domains, including a C-terminal end of the protein where a large majority of mutations that contribute to the disease are located. Due to FUS' aggregation prone nature, it is speculated that motor neuron death seen in ALS is due to a loss of function or a toxic gain of function. However, we cannot determine the effect of these aggregates on cellular function without first understanding their biochemical make up.

Objectives: In this study we hypothesized that FUS inclusions sequester splicing proteins and small nuclear riboproteins (snRNPs), impairing mRNA metabolism. This may contribute to the splicing abnormalities reported in the literature.

Methods: Traditional methods are unable to isolate stable aggregates and have limited our ability to analyze these structures. Earlier experiments to explore the mutant FUS interactome employed immunoprecipitation techniques, which limited the interacting species that could be pulled down. Here we use low detergent containing lysis buffer and membrane filtration methods to capture FUS aggregates for high performance liquid chromatography (HPLC) and mass spectrometry (LC MS/MS). Using this approach we are able to fully survey the protein composition of the aggregates without disrupting their delicate structure. This provides us with a better understanding of the mutant FUS interactome, something that has not yet been described in the literature.

Results: Our data suggests that these captured aggregates directly sequester critical splicing proteins, such as U2AF2 and U5-200 snRNP. FUS is known to interact with several proteins that regulate the splicing cascade, further dampening the cell's ability to alternatively splice pre-mRNA transcripts (4). In accordance with these findings, our evidence suggests that a loss of splicing ability is at

least partially due to sequestration of critical splicing proteins by aberrant FUS aggregates.

Discussion and conclusions: This data provides mechanistic support for recent publications demonstrating defective splicing in cells containing FUS mutants. RNA also structurally supports these aggregates, as demonstrated by the addition of nucleases to aggregate containing cell lysate. Overall, we can conclude that these FUS aggregates observed in clinical and experimental models sequester RNA splicing proteins and potentially RNA. These RNA molecules are required for their structural integrity and provide us with future experimental opportunities.

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P212 AN EMERGING ROLE OF FUS AT THE NEUROMUSCULAR JUNCTION: IMPLICATIONS FOR ALS PATHOGENESIS

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Keywords: FUS, transgenic mouse model, neuromuscular junction

Background: FUS mislocalization and cytoplasmic aggregation are hallmark pathologies in some ALS and FTD cases. Mechanistic hypotheses have largely focused on the nuclear functions of FUS in transcriptional regulation and splicing. Recent reports describe a role for FUS in RNA axonal transport and its presence in dendritic spines. Mice transgenic for wild-type human FUS overexpression develop a progressive and ultimately fatal motor deficit due to motor neuron degeneration (1). We sought to explore the peripheral role of FUS at the neuromuscular junction (NMJ).

Objectives: To determine the peripheral distribution of neuronal FUS and describe the earliest pathology and temporal progression of motor neuron degeneration.

Methods: Immunohistochemistry for FUS and a range of synaptic proteins and gene expression analyses were performed on the spinal cord and NMJ of the gastrocnemius muscle of WT-hFUS$^{+/+}$, WT-hFUS$^{+/-}$ and non-transgenic mice, at pre-symptomatic (post-natal days 6 and 15) and endstage (10-12 weeks) disease. To define changes in the ultrastructural morphology of the NMJ, electron microscopy and immunogold labeling of FUS were used.

Results: FUS is highly abundant at the NMJ in both non-transgenic and transgenic mice, implicating a significant peripheral function. The pre-synaptic protein synaptophysin was lost, with evidence in early pre-symptomatic (P15) WT-hFUS$^{+/+}$ mice accompanied by morphological abnormalities at the NMJ without evidence of any morphological change to motor neurons in the spinal cord. Electron microscopy revealed early ultrastructural changes at the NMJ, including a dramatic loss of synaptic vesicles and mitochondrial abnormalities in the pre-synaptic terminals at P15. However, cresyl violet staining and cell counting indicated no loss of spinal cord motor neurons in WT-hFUS$^{+/+}$ mice compared with non-transgenic littermates.

Discussion and conclusion: The abundance of FUS at the NMJ indicates an important peripheral function in RNA processing. FUS overexpression causes major disruption of the NMJ without significant motor neuron loss from the spinal cord pre-symptomatically, consistent with a dying back disease mechanism in which the impaired peripheral function of FUS may cause synaptic and axonal dysfunction leading to motor neuron degeneration in ALS.

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P213 DENERVATION OF ALS NEUROMUSCULAR JUNCTIONS IS CAUSED BY ACTIVE ZONE LOSS AND IS AMELIORATED BY EXERCISE

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Keywords: synapse, denervation, exercise

Background: ALS neuromuscular junctions (NMJs) denervate before the degeneration of motor neuron cell bodies suggesting a dying back neuropathy. However, the mechanism of NMJ denervation remains unknown.

Objectives: The object of this study is to elucidate the mechanism of NMJ denervation in ALS and to seek interventions to ameliorate the denervation.

Methods: NMJs of $SOD1^{G93A}$ mice and rats were analyzed using confocal microscopy and transmission electron microscopy to detect synaptic defects from presymptomatic to symptomatic stages (postnatal day 40 and 140). $SOD1^{G93A}$ rats were exercised unilaterally using our isometric force training method. $SOD1^{G93A}$ mice were exercised using running wheels for aerobic exercise and inverted screen training for resistance exercise. Transgenic mice expressing laminin β2 in skeletal muscles were mated to $SOD1^{G93A}$ mice to test the role of laminins in synapse maintenance.

Results: Here we show that a loss of active zones (synaptic vesicle release sites) is a cause of NMJ denervation in ALS model mice. In the presymptomatic stage of $SOD1^{G93A}$ mice, innervated NMJs showed a significant loss of active zones detected by electron microscopy and a decreased level of active zone protein Bassoon detected by confocal microscopy. Previously, we identified a molecular mechanism that organizes NMJ active zones involving active zone organizer laminin β2, a specific receptor for laminins, and active zone specific proteins (1). Consistent to this molecular mechanism, the protein level of active zone organizer laminin β2 was significantly decreased in ALS NMJs that had reduced number of active zones. Chronic loss of active zones caused denervation of NMJs in mice suggesting an essential role of active zones in maintaining NMJ innervation in ALS.

Importantly, exercise restored the laminin β2 protein level and active zone number at NMJs, ameliorated NMJ denervation, and improved neuromuscular functions of $SOD1^{G93A}$ mice and rats. Interestingly, resistance exercise had stronger beneficial effect over voluntary aerobic exercise for innervation. Finally, transgenically expressed laminin β2 protein ameliorated NMJ denervation in $SOD1^{G93A}$ mice without exercise, suggesting that laminin β2 protein level at NMJs plays a key role for the exercise induced mechanism that maintains NMJs.

Discussion and conclusions: Current results suggest the potential use of exercise as a non-pharmacological intervention to maintain motor function in ALS.

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P214 THE EFFECT OF SOD1 ZINC LOSS ON THE INTERACTION WITH HCCS

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Keywords: SOD1, hCCS, protein complex

Background: ALS SOD1 mutations are destabilizing and promote aggregation. Mutation dependent decrease of SOD1 affinity for zinc is a potential cause of these deleterious characteristics. The high chelation capacity of the zinc-binding proteome will divert this highly utilized cofactor to other cellular destinations, leaving a portion of SOD1 monomers destined to never bind zinc. These properties manifest as accumulation of metal depleted, disulphide reduced SOD1 in cytoplasmic aggregates prior to neuronal death. While zinc metalated disulphide reduced SOD1 is the substrate for hCCS, apoSOD1 is competent to interact with hCCS by formation of the heterodimer complex.

Objective: To characterize the effect of normal, or lack of normal, SOD1 post-translational modifications on the functional interaction between SOD1 and its chaperone hCCS.

Methods: Recombinant SOD1 was modified by removing metal cofactors. The formation and dissolution of the heterodimer complex formed between copper (I) loaded hCCS and SOD1 was then observed over a time course.

Results: The apoSOD1-hCCS heterodimer is larger than the ZnSOD1-hCCS counterpart consistent with destabilization of the SOD1 zinc-binding loop. Disulphide reduced SOD1 forms a complex with Cu(I)-hCCS that displays exponential decay and reformation of homodimeric species. By contrast, demetalated SOD1 forms a complex with Cu(I)-hCCS that resists dissolution. This is the case for wild-type SOD1 and several ALS mutants.

Discussion and conclusion: Our findings illustrate that zinc deficiency inhibits hCCS-SOD1 heterodimer complex dissociation. Therefore, compromised zinc binding in ALS SOD1 mutants likely prevents disulphide oxidation, traps hCCS in a non-productive complex and reduces the availability of hCCS to interact with newly translated SOD1. In this situation, both stabilizing SOD1 post-translational modifications are compromised and copper metalation is reduced. This hypothesis offers an explanation for the presence of disulphide-reduced SOD1 in the CNS tissue of mutant transgenic models.

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P215 DISCREET UNFOLDED SPECIES OF SOD1 ARE REVEALED BY NATIVE MASS SPECTROMETRY

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Keywords: SOD1, structure, Mass Spectrometry

Background: Mutations in the enzyme superoxide dismutase-1 (SOD1) are linked to familial amyotrophic lateral sclerosis. These mutations destabilise the protein structure leading to unfolding/misfolding and aggregation. SOD1 stability is dependent upon its post-translational modifications (Cu/Zn incorporation and the intramolecular disulphide), which can be perturbed by mutation. Their disruption is required for the *in vitro* formation of aggregates. Understanding protein misfolding and aggregation has been difficult, as inducing these events leads to the formation of structurally heterogeneous samples composed of variable tertiary and quarternary states. This heterogeneity makes classical techniques of structural analysis, such as x-ray crystallography, problematic. It is especially true for early unfolding and aggregation events as they are transient. Native mass spectrometry is becoming increasingly prevalent as a tool for the structural analysis of proteins, allowing for the separation of samples based upon their mass-to-charge ratio, and also their drift-time when coupled with ion-mobility analysis (1). These techniques can be used to probe important information from structurally heterogeneous samples, such as oligomeric distribution, spatial topology, and protein-protein dynamics.

Objectives: The aim of our work is to investigate the unfolded intermediate states that SOD1 can potentially occupy when destabilised through mutation, metal loss, and disulphide reduction.

Methods: We treated purified recombinant SOD1 variants with EDTA and/or DTT to induce unfolding. We analysed these samples using native-PAGE, analytical gel-filtration chromatography, and native mass spectrometry coupled with an ion-mobility analyser.

Results: Native mass spectrometry revealed species that could not be resolved using native-PAGE or analytical gel-filtration chromatography. All SOD1 variants analysed showed differential destabilisation based on the presence of non-native species in their respective mass spectra. A common extensively unfolded species was observed for all SOD1 variants. Ion-mobility mass spectrometry showed that, after EDTA and/or DTT treatment, the spatial conformations of both the monomeric and dimeric forms of our SOD1 variants were altered compared to the native conformation. Also, we were able to acquire mass spectra of early oligomeric species composed of dimers to decamers for $SOD1^{G37R}$.

Discussion: Native mass spectrometry was able to resolve the aggregating mix into its constitutive conformations, revealing the effects of disulphide reduction and metal chelation on SOD1. We attribute the difference in unfolded SOD1 abundances, between variants, to differential susceptibility to destabilisation by DTT and/or EDTA. This variable susceptibility has been observed before using native-PAGE (2), but here we show fully resolved mass spectra of the species generated from destabilising several disease associated SOD1 variants. We propose that this variable susceptibility to the loss of post-translational modifications (and hence variable aggregation propensity) may account for the difference in disease progression in patients suffering from SOD1 associated fALS.

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P216 MUTANT SOD FACILITATES NITRATION OF HSP90

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Keywords: SOD, Hsp90, nitrotyrosine

Mutations in the superoxide dismutase gene trigger ALS pathology by a toxic gain of function. Here we show that nitrated Hsp90, an oxidative modification that induces motor neuron death, occurs during disease onset and progression and that mutant SOD facilitates Hsp90 nitration.

Nitrated Hsp90 was detected by immunohistochemistry in spinal motor neurons during symptom onset in transgenic mutant SOD animal models but not in mice overexpressing wild type SOD as well as non-transgenic controls. Nitrated Hsp90 staining was specific to motor neurons and the levels increased with disease progression. Analysis of ventral spinal cord by western blot and ELISA confirmed the presence of nitrated Hsp90 at symptom onset and during disease progression.

Mutant SOD (D85S –Zn-deficient SOD- and H46R/H48Q/H63G/H120G -Quad SOD-) but not wild type enzyme, added together with peroxynitrite to brain homogenates from non-transgenic animals induced nitration of Hsp90. Surface plasma resonance showed that Hsp90 directly binds Zn-deficient and Quad SOD but not the wild type enzyme. Both, Zn-deficient and Quad SOD induced motor neuron death 24 h after intracellular delivery of the enzymes. Mutant SOD toxicity was prevented by addition of nNOS inhibitors, peroxynitrite scavengers and copper chelators. Moreover, the toxicity of quad and zinc-deficient SOD correlated with the presence of nitrated Hsp90 in motor neurons.

Importantly, a copper depleted SOD (C111S E,Zn) was not toxic to motor neurons, suggesting that copper is indeed required for mutant SOD toxicity. These findings reveal that mutant SOD facilitates Hsp90 nitration leading to motor neuron death.

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P217 REGIONAL- AND LAMINA-SPECIFIC ALTERATIONS IN CALRETININ AND NPY INTERNEURON POPULATIONS IN THE SOD1 MICE AND AMYOTROPHIC LATERAL SCLEROSIS PATIENTS: A POTENTIAL SOURCE FOR CORTICAL HYPEREXCITABILITY

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Keywords: interneuron, hyperexcitability, mSOD1

Background: In amyotrophic lateral sclerosis (ALS), hyperexcitability of cortical motor circuitry is a prominent event, often preceding motor neuron degeneration. Whilst many factors may be attributed to this pathophysiology, a possible candidate, in the interneuron, has largely been overlooked.

Methods: Here, in a systematic immunohistochemcical study of interneuron subsets and morphologies we demonstrate unique regional- and lamina-specific alterations to specific populations in human end-stage ALS (n=6 cases and controls) and in $SOD1^{G93A}$ transgenic mice. Investigations are performed in motor and control somatosensory cortex from presymptomatic stages through to end-stage (n=6, for each timepoint).

Results: In SOD1 mice we find that in late symptomatic stages, specific interneuron subsets of the motor cortex display contrasting (and potentially complimentary) pathology; the density of calretinin (CR) populations is significantly reduced by 37% in supragranular lamina (WT, n=4, $55.3 \pm 6.9 mm^2$; SOD1, n=4, $35.3 \pm 6.0 mm^2$), whilst neuropeptide Y (NPY) populations are increased by 40% in the infragranular lamina (WT, n=4, $18.6 \pm 2.4 mm^2$; SOD1, n=4, $31.0 \pm 4.2 mm^2$) ($p < 0.05$, two-way ANOVA with Bonferroni post-hoc test). These alterations were not present in the somatosensory cortex, nor were they in other interneuron populations (parvalbumin, vasoactive intestinal peptide and somatostatin). Furthermore, using morphometric approaches, we show that CR interneurons in $SOD1^{G93A}$ tissue undergo early, and continuing alterations to their neurite morphology – with progressive reductions in branch complexity from presymptomatic- to late-symptomatic stages. Analysis of human ALS post-mortem brain tissue revealed a cluster of ALS cases with reduced CR density in lamina II/III compared with controls.

Discussion and conclusions: This study provides evidence for altered inhibition in ALS and reveals specific interneuron subclasses likely to contribute to this pathophysiology. Our findings indicate that inhibitory regulation of cortical circuitry is impaired in a region- and lamina-specific manner, due to changes in Calretinin and NPY interneurons, prior to, and potentially contributing to, motor neuron loss in ALS.

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P218 POST¬TRANSLATIONAL MODIFICATIONS PROMOTE FORMATION OF SOD1 OLIGOMERS WITH POTENTIAL TOXICITY IN ALS

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Keywords: post-translational modifications, oligomers, thermodynamic stability

Background: Mutation of the ubiquitous cytosolic enzyme Cu/Zn superoxide dismutase (SOD1) is associated with a subset of cases of familial ALS (FALS) through destabilization of SOD1, leading to misfolding and aggregation. The sporadic nature of most ALS cases and the late onset of symptoms, even for patients expressing disease-linked mutant proteins throughout life, suggest a potentially significant role of environmental factor(s) in ALS etiology. We have previously determined that SOD1 from human tissue is extensively post-translationally modified, with the most prevalent modifications being glutathionylation at Cys-111 and phosphorylation at Thr-2. We found that glutathionylation significantly destabilizes $SOD1^{WT}$ dimers, increasing the equilibrium dissociation constant approximately 1,000-fold, such that its stability is comparable to the A4V FALS-causative mutation. $SOD1^{A4V}$ is destabilized by glutathionylation even further.

Objectives: Determine whether oxidative stress-induced glutathionylation promotes the formation of toxic oligomers.

Methods: We employ computational structural modeling combined with a broad range of biophysical and biochemical experiments.

Results: We show that glutathionylation has distinct effects on different ALS-linked SOD1 mutants, which correspond to changes in composition of the dimer interface. Glutathionylation also stabilizes an intermediate misfolded species in which the b-barrel core of each monomer is "loosened". We demonstrate the formation of soluble oligomers at the early stages of SOD1 aggregation. These oligomers feature an epitope specific to disease-relevant misfolded SOD1, which is recognized by the C4F6 antibody (a marker of toxic species).

Discussion and conclusions: These findings suggest that soluble non-native SOD1 oligomers, rather than native-like dimers or monomers, share structural similarity to pathogenic misfolded species found in ALS patients and therefore represent potential cytotoxic agents and therapeutic targets in ALS.

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P219 SOD1 TRANSMISSION TO MOTOR NEURONS IN A SOD1G85R MOUSE AMYOTROPHIC LATERAL SCLEROSIS (ALS) MODEL

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Keywords: spread, SOD1, chimeric mice

Background: Transmission of aggregation-prone proteins between neurons has been hypothesized to contribute to neurodegeneration in patients and mouse models. Mutations in superoxide dismutase 1 (SOD1) cause approximately 20% of familial ALS cases and cause protein aggregation in motor neurons in the spinal cord. Motor neuron demise in ALS patients is thought to be due to a toxic gain-of-function as a result of SOD1 misfolding. SOD1 aggregates can be taken up by dividing cells in culture (1) and injection of spinal cord lysate from diseased $SOD1^{G93A}$ transgenic mice into the spinal cord of newborn $SOD1^{G85R}$-YFP mice can accelerate ALS pathogenesis (2). These data raise the question of whether mutant SOD1 can be taken up by motor neurons in the spinal cord *in vivo*, thereby causing disease spread.

Objectives: To assess whether mutant SOD1 spreads between cells in mouse spinal cord

Methods: We have modeled SOD1-linked ALS using transgenic mice expressing $SOD1^{G85R}$-YFP. $SOD1^{G85R}$-YFP mice develop large, cytosolic, YFP-fluorescent aggregates in motor neurons and are paralysed by six months of age, while $SOD1^{WT}$-YFP mice do not develop aggregation or motor neuron disease symptoms. To visualize SOD1 propagation in motor neurons, we generated a second transgenic strain expressing $SOD1^{G85R}$-CFP. Chimeric mice were formed from embryos of the two $SOD1^{G85R}$-FP strains resulting in spinal cord neurons expressing one or the other fluorescently labeled mutant SOD1. Transfer of mutant SOD1 between neurons would result in cells co-labeled by both the endogenously expressed mutant SOD1 and SOD1 derived from cells expressing the other fluorescently tagged mutant.

Results: While no mutant SOD1 is transferred between neurons at early time points (1 month), small amounts of SOD1 can be transmitted preferentially to spinal motor neurons at later time points (3 months) in symptomatic, SOD1 chimeric mice. Motor neurons in trigeminal, facial, and hypoglossal motor nuclei can also acquire the second mutant SOD1, while uptake in motor neurons in the oculomotor, trochlear, and abducens nuclei is not detectable at 3 months.

Conclusions: These results are a proof-of-principle that a predominantly cytosolic protein can exit motor neurons and be taken up by other motor neurons *in vivo*, a necessary step if misfolded SOD1 is to template aggregation in recipient cells. These data support the need for further studies to address the mechanism and pathogenicity of SOD1 protein transfer, as interruption of this process may comprise a therapeutic avenue in ALS patients.

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P220 TDP-43 OR FUS-INDUCED MISFOLDED WILD-TYPE SOD1 CAN SPREAD INTERCELLULARLY AND INDUCE SOD1 MISFOLDING IN RECIPIENT CELLS

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Keywords: SOD1, protein misfolding, propagation

Background: Clinically indistinguishable cases of ALS are caused by either inheritable mutations in genes such as SOD1, TDP-43, FUS, or can occur sporadically. Misfolded SOD1 has been detected in all ALS patients (1-3), despite SOD1 mutations accounting for only 2% of total cases, while the presence of pathological TDP-43 or FUS is a hallmark of all non-SOD1 familial ALS. We previously reported that pathological TDP-43 or FUS are associated with the misfolding of human wild-type (wt) SOD1 in living cells (1), but its role in sporadic ALS is unknown.

Objective: To test the hypothesis that TDP-43 or FUS-induced misfolded wtSOD1 can propagate between cells and induce cytotoxic SOD1 misfolding in the recipient cells.

Methods: We generated conditioned media containing misfolded wtSOD1 by transfecting HEK293 cells, which was placed on either primary mouse spinal cord cultures from human wtSOD1 transgenic mice, or on fresh HEK293 cells. To identify misfolded SOD1, we performed quantitative immunoprecipitation and

immunofluorescence studies using SOD1 misfolding-specific antibodies (1, 4). Blocking of intercellular transmission of misfolded SOD1 was performed using SOD1 misfolding-specific antibodies (5). To study the role of endogenous wtSOD1 as substrate for conversion, we used SOD1-siRNA to knockdown SOD1 expression prior to incubation with conditioned media. For cytotoxicity, we used DHE to detect superoxide radicals, and MTT to establish cell viability.

Results: We show that pathological TDP-43 or FUS-induced misfolded SOD1 can traverse between cells through the incubation of untransfected cells with conditioned media, triggering new rounds of wtSOD1 misfolding. This spread of SOD1 pathology is cytotoxic to the recipient cells as determined by increased oxidation and lower cell viability. Knockdown of SOD1 in recipient cells prior to incubation with conditioned media prevents the spread of SOD1 pathology, indicating that endogenous SOD1 itself is required as substrate for active conversion. We can arrest the intercellular spread of protein misfolding by pre-incubating the conditioned media with SOD1 misfolding-specific antibody. Furthermore, transfection of TDP-43 into cells triggers its cleavage, mislocalization and hyperphosphorylation; these findings are not observed in cells incubated with conditioned media from TDP-43 transfected cells, further confirming that the transmission of SOD1 misfolding occurs independently of TDP-43.

Conclusion: Pathological TDP-43 and FUS can trigger the misfolding of wild-type SOD1, which subsequently propagates from cell to cell and induces additional rounds of cytotoxic protein misfolding. Additionally, SOD1 misfolding-specific antibody can arrest the spread of SOD1 pathology, consistent with therapeutic potential not only in SOD1-ALS but also in all non-SOD1 ALS.

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P221 SPINE LOSS IS AN EARLY PATHOGENIC EVENT IN THE TDP-43 A315T MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: TDP-43, synapse, spines

Background: The RNA binding protein TDP-43 is the major component of inclusions defining ALS pathologically and the gene encoding TDP-43 has been identified as a genetic cause for ALS, highlighting the importance of this protein. Whilst previous research has focussed on the normal role of TDP-43 in the nucleus and cytoplasmic aggregates pathologically, recent research has indicated that TDP-43 mis-processing, as a consequence of either TDP-43 mislocalisation or TDP-43 mutant gain/loss of function, may have an underappreciated pathological role at the synapse

Objectives: Characterise the pre- and post-synaptic pathology occurring in the TDP-43^{A315T} mouse model of ALS with regional immunohistochemistry (IHC), western blots and spine density analysis. Spine analysis and spinal tract tracing investigated in TDP-43^{A315T} YFP-H fluorescent mice.

Methods: Mice were perfused with either 4% paraformaldehyde or 0.1M PBS over a time-course of disease (postnatal day 30 to day 90). Protein expression in the cortex was investigated using western blots and immunohistochemistry at day 90 with antibodies directed at pre- and post-synaptic markers. Dendritic spines and axonal tracts in the TDP-43^{A315T}YFP-H mice were investigated in 20μm coronal sections and 40μm spinal sections, respectively, on the Zeiss LSM 510 Meta confocal microscope and using Neurolucida software ($p<0.05$ considered significant).

Results: At postnatal day 90 (P90) there was a significant reduction in the total number of YFP positive pyramidal neurons in the motor cortex of TDP-43^{A315T} YFP-H mice, in comparison to YFP-H-controls (71 ± 12/ηm TDP43; 168 ± 29/ηm YFP-H-control). At this time point there was a significant reduction in total spine density in the motor cortex of TDP-43^{A315T} YFP-H mice, in comparison to YFP-H-controls. These alterations were not associated with any significant changes in the glial cell (Iba1 and GFAP), neuronal pre-synaptic (synaptophysin, VGLUT1, VGULT2 and VGAT1) or post-synaptic (GluR1-4) protein expression. However, they were associated with axonal tract degeneration in the corticospinal tract of the spinal cord. Furthermore we found that the dendritic spine alterations were an early pathogenic event, developing between postnatal day 30 and 60 (P60), with a reduction in somatodendritic spine density in the motor cortex of TDP-43^{A315T} YFP-H mice at P60, in comparison to YFP-H-controls (632.4 ± 7/ηm TDP-43; 855.2 ± 11/ηm YFP-H-control), prior to any significant YFP positive cell loss in the TDP-43^{A315T} YFP-H motor cortex.

Discussion and conclusions: The data suggests that morphological alterations to somatodendritic spine densities mediated by TDP-43 mutations may be an early disease-related event in ALS. Further research is needed to elucidate the mechanism behind these spine alterations, and to determine whether these are an upstream or a downstream pathological event in the motor neuron degeneration that characterises this devastating disease.

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P222 PHOSPHORYLATION OF HNRNP K BY CYCLIN-DEPENDENT KINASE 2 CONTROLS CYTOSOLIC ACCUMULATION OF TDP-43

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Keywords: TDP-43, CDK-2, hnRNP K

Background: Tar DNA binding protein 43 kDa (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. hnRNPs have gained considerable attention over the years as mutations in genes encoding these proteins have been linked to the pathogenesis of ALS. hnRNPs including TDP-43, hnRNPA1, and hnRNPA2/B1 have been identified to localise in RNA stress granules (SGs) in the disease state. The potential role for TDP-43 SG localisation as a key step in ALS and FTLD-U neuropathology has led to major efforts to understand the mechanisms controlling TDP-43 accumulation in SGs. Movement of hnRNPs into SGs has been reported to be regulated by kinases. Phosphorylation of hnRNPs by kinases may be a central factor in driving neurodegeneration in ALS by altering the physiological components of the hnRNPs and affecting protein–protein interactions, which may have deleterious effects pathologically.

Objective: Here we used cell models and human ALS spinal cord motor neurons to investigate how Cyclin dependent kinase-2 (CDK2) modulates the accumulation of TDP-43.

Results: In the present study, we investigated the relationship between TDP-43 and hnRNP K and their control by CDKs. We found that selective inhibitors of CDK2 abrogated the cytosolic accumulation of TDP-43. Inhibition of CDK2 phosphorylation blocked phosphorylation of hnRNP K, preventing its incorporation into SGs. Confirmation of the importance of hnRNP K in TDP-43-positive SG formation was obtained through mutation of key phosphorylation sites (Ser216 and Ser284) on hnRNP K, this inhibited hnRNP K and TDP-43 colocalisation to SGs. siRNA-mediated inhibition of hnRNP K expression attenuated TDP-43 positive SGs. A substantial decrease in CDK2 and hnRNP K expression in spinal cord motor neurons from ALS patients was evident.

Discussion and conclusion: Our recent studies have identified a key role for specific kinases controlling cytosolic accumulation of TDP-43. While TDP-43 does not contain known phosphorylation consensus sites, our data strongly suggest that a key process in control of TDP-43 trafficking involves phosphorylation of hnRNP K by CDK2 at Ser216 and/or Ser284 during cell stress. Our findings are the first to describe a prominent change to hnRNP K and CDK2 in human ALS spinal cord tissue. This demonstrates a potential key role for these proteins in ALS and TDP-43 accumulation. Understanding how kinase activity modulates TDP-43 accumulation may provide new pharmacological targets for disease intervention.

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P223 DEPLETION OF TDP-43 INDUCES MITOCHONDRIAL FRAGMENTATION

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Keywords: opa1, mitochondria, dynamics

Background: Mitochondria are movable organelles and build up a large network called mitochondrial dynamics by their fusion and fission. Mitochondrial dysfunction has been reported as a molecular pathogenesis of neurodegenerative diseases including amyotrophic lateral sclerosis (ALS). Abnormal mitochondrial morphology and fragmentation have been detected in motor neurons with ALS. However, the molecular background for these findings are still obscure. Dislocation of nuclear protein TDP-43 to cytoplasm and the formation of inclusions is a pathological hallmark of ALS. TDP-43 has been shown to have multiple functions in transcriptional repression, pre-mRNA splicing and translational regulation. A loss of function of TDP-43 is thought to be a key process for the molecular pathogenesis of ALS. Here, we investigated whether a loss of TDP-43 affects mitochondrial morphology.

Method and result: We found that the depletion of TDP-43 by siRNA induced mitochondrial fragmentation in HEK293T cells. Using Western Blotting, we investigated the amounts of mitochondrial dynamics related proteins: DRP1, FIS1, OPA1, MFN2. Among them, OPA1 regulates the fusion of mitochondrial inner membrane, and its 87 kDa protein significantly increased under

the depletion of TDP-43. OPA1 has eight mRNA splicing variants, with each of them undergoing cleavage to finally produce five peptides. We investigated the amounts of each splicing variant by qPCR and found that variant 8, which produces a 87 kDa peptide, was increased following the depletion of TDP-43. Additionally, the amounts of OPA1 mRNA exons 4, 4b and 5b, which are involved in alternative splicing, were investigated; exon 4b, which are included in variant 8, significantly increased. Finally, we found overexpression of OPA1 variant 8 resulted in the mitochondrial fragmentation and increased expression of OPA1 87 kDa.

Conclusion: The depletion of TDP-43 induced mitochondrial fragmentation and increased levels of the OPA1 splicing variant 8, which produces OPA1 87 kDa peptide. OPA1 mRNA splicing regulates mitochondrial fusion, and OPA1 variant 8 doesn't have an ability of mitochondrial fusion. Furthermore, OPA1 exon 4b, a key exon that regulates OPA1 protein cleavage, increased in TDP-43 depletion cells. Increased exon 4b would disturb the balance between long and short OPA1. This perturbation of OPA1 splicing variants induced by TDP-43 depletion may associate with the mitochondrial fragmentation.

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P224 PTDP-43 STAGING OF AMYOTROPHIC LATERAL SCLEROSIS IN OUR 27 CASES

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Keywords: pTDP-43, staging,

Background: Phosphorylated (p) TDP-43 is a major component of neuronal cytoplasmic inclusions (NCI) in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). Stages of pTDP-43 pathology in ALS have been described (1). In addition, two pathological patterns of TDP-43 immunoreactive neuronal cytoplasmic inclusions (NCIs) and glial cytoplasmic inclusions (GCIs) of ALS have also been documented (2).

Objective: To observe the distribution patterns of pTDP-43 in ALS patients.

Methods: We examined 27 ALS cases from consecutive autopsy brains between August 1995 and October 2013. Sections of agranular motor cortex (Stage 1), medulla oblongata (Stage 2), prefrontal cortex and striatum (Stage 3), hippocampus (Stage 4) were immunostained with pTDP-43 antibody (PSer409/410). The immunoreactive structures were classified into NCI or GCI and dystrophic neurite (DN).

Results: pTDP-43 pathology in this study was distinguished as follows; stage 1 (n = 2), stage 2 (n = 3), stage 3 (n = 4), stage 4 (n = 11), unclassifiable (n = 7). In unclassifiable cases, two cases were type 1 and 5 cases were type 2 (2). Two cases showed none, or only small numbers of pTDP-43 immunoreactive NCIs/GCIs in all regions. Three cases showed only small numbers of pTDP-43 immunoreactive NCIs/GCIs in the agranular motor cortex and/or medulla oblongata. One case did not show pTDP-43 immunoreactivity in the striatum, but in the hippocampus. Another case did not show pTDP-43 immunoreactivity in the prefrontal cortex, but in the striatum and hippocampus.

Discussion and conclusions: We found 26% (7/27) unclassifiable cases in this study. This finding suggests that there is a group taking a different distribution pattern from the pTDP-43 stages previously described. Our study included patients of long duration who had severe neuronal loss. There are cases lacking pTDP-43 pathology in the prefrontal cortex or striatum and sequential patterns of TDP-43 propagation may not be a single pathway.

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P225 A CERTAIN MORPHOLOGY OF ACTIVATED MICROGLIA, WHICH CORRELATES WITH TDP-43 PATHOLOGY IN ALS SPINAL CORD

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Keywords: microglia, anterolateral funiculus, inflammation

Background: Microglia (Mi) in the CNS can exhibit several morphologies during activation. We previously reported massive infiltrations of activated Mi in the anterolateral funiculus in amyotrophic lateral sclerosis (ALS) spinal cords; however, the relationship of Mi morphological differences to motor neuron impairment remains unknown.

Objectives: We aimed to clarify which morphology and location in the spinal cord of Mi correlates with TDP-43 pathology in ALS patients.

Methods: Ten-percent buffered formalin-fixed, paraffin-embedded 5-μm-thick transverse spinal cord sections of sporadic ALS cases (n=7) and non-ALS cases (n=5) were examined by immunohistochemical methods using primary antibodies against Iba-1, CD68, SMI-31, iNOS, GDNF, and TDP-43. The numbers of immunoreactive (ir) cells in the corticospinal tracts (CST), anterolateral funiculus outside the CST (ALFoc), and anterior horns (AH), those of motor neurons with TDP-43 pathology in the AH, and those of axons in the CST and ALFoc' were quantified for correlation analyses. Morphological alterations of Iba-1- or CD68-ir cells were classified into "foamy" and "non-foamy" appearance.

Results: Only the number of Iba-1-ir cells with foamy appearance in the ALFoc of ALS patients showed a positive correlation with TDP-43 pathology in the AH (r=0.673, p=0.0060). Most of these cells were iNOS-ir, and occasionally, GDNF-ir. There was a tendency toward negative correlation between numbers of Iba-1-ir cells showing non-foamy appearance and TDP-43 pathology (r=-0.506, p=0.054). Otherwise there were no significant correlations between TDP-43 pathology and Iba-1-ir/CD68-ir cells irrespective of their morphologies or locations. Although there were no correlations between decrease in axon numbers and TDP-43 pathology, distinct reductions of axons in the both CST and ALFoc were found in ALS cases. Morphology of Iba-1-ir cells in the AH was always non-foamy, and thicker in ALS cases as compared with non-ALS cases.

Discussion and conclusions: The present study first suggests that Mi with foamy appearance in the ALFoc, but not the AH or CST, correlate to TDP-43 pathology of the motor neurons in ALS spinal cords, providing an insight for novel therapeutic targets in this disorder. It is hypothesized that Mi with foamy appearance might reflect phagocytosis, while Mi with non-foamy appearance might reflect synaptic stripping.

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P226 DETERMINE THE CONTRIBUTION FROM SENSE VERSUS ANTISENSE TRANSCRIPT TO C9ORF72 HEXANUCLEOTIDE REPEAT EXPANSION-MEDIATED TOXICITY

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Keywords: C9ORF72, RNA profile, antisense oligonucleotide (ASO)

Background: Hexanucleotide repeat expansion in the C9orf72 gene is the most common genetic cause of both amyotrophic lateral sclerosis (ALS) and frontal temporal dementia (FTD). The genetic region is bi-directionally transcribed, making both sense (GGGGCC)n and antisense (CCCCGG)n repeat RNAs. Both transcripts form RNA foci that could sequester RNA binding proteins and disrupt RNA processing, and produce dipeptide repeat proteins (DPRs) by repeat-associated non-ATG translation (RAN translation).

Objectives: To determine how exactly, or to what extent, each transcript strand contributes to disease pathogenesis.

Methods: We define genomic RNA signatures linked to C9orf72 expansion using neurons directly trans-differentiated from multiple control and C9orf72 patient fibroblast lines, and following expression of either sense or antisense repeats in normal cells by high-throughput RNA sequencing. We also treat the fibroblast converted neurons with antisense oligonucleotides (ASOs) to reduce either sense or antisense transcript to examine the relative contribution from each strand to the overall C9orf72-linked RNA profile.

Results: Principle component analysis of gene expression changes showed that neurons from C9orf72 patients are separated from controls, defining an RNA profile linked to C9orf72 expansion. Up-regulation and down-regulation of selected genes were confirmed by quantitative RT-PCR in neurons derived from patient fibroblasts. The relative contributions from sense versus antisense repeats were investigated in patient neurons by analyzing reversal of RNA changes linked to C9orf72 hexanucleotide expansion after reducing either RNA strand with sense or antisense RNA targeting antisense oligonucleotides (ASOs). We determine that reducing repeat-containing RNAs from the sense strand is not sufficient to correct RNA alterations and identify RNA expression changes dependent on the expression of the antisense (CCCCGG)n repeat RNAs.

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P227 BUNINA BODIES IN C9-ALS NERVOUS SYSTEMS

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Keywords: C9orf72, neuropathology, Bunina bodies

Background: Bunina bodies are small, round/oval eosinophilic intracellular inclusions in the cytoplasm of

motor neurons of the spinal cord and brain stem of both sporadic and familial ALS patients, best seen with Hematoxylin and Eosin (H and E) (1). Since the most recent comprehensive studies of Bunina bodies, a large body of research has been published about ALS pathogenesis. It is therefore necessary to examine Bunina body pathology in light of our current genetic and molecular knowledge of ALS. In particular, it was discovered in 2011 that an intronic GGGGCC hexanucleotide repeat expansion in C9orf72 was responsible for over 20% of familial ALS cases, making it the most common known genetic cause of ALS (2,3). To date, no studies have examined the prevalence or distribution of Bunina bodies in C9orf72 ALS patients. Doing so could improve our understanding of the link between Bunina bodies and ALS.

Objectives: To determine and compare the burden and distribution of Bunina bodies in cases of sporadic ALS and familial ALS, including patients harboring SOD1 mutations and C9orf72 repeat expansions.

Methods: Hematoxylin and eosin staining of the cervical, thoracic, and lumbar spinal cord of 13 ALS patients (5 C9orf72, 3 SOD1, and 5 sALS) matched for age and site of disease onset, and five age-matched controls. For each patient, three sections of each spinal cord region were stained with H&E. The number of motor neurons in the anterior horns of each section was quantified, as was the number of Bunina bodies found in each neuron.

Results: Bunina bodies are observed in C9-ALS spinal cord sections. Quantification is ongoing to determine the relative burden of Bunina bodies in C9-ALS versus sporadic ALS and SOD1-ALS. However, preliminary data suggests there is no significant difference in Bunina body pathology between C9-ALS and sALS.

Discussion and conclusions: Our preliminary findings show comparable frequency and distribution of Bunina bodies in C9-ALS and sALS patients. This suggests that the mechanism leading to Bunina body pathology is common to ALS regardless of underlying genetics. Further effort should be made to understand the mechanisms leading to Bunina body formation and how it relates to ALS.

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P228 ALS/FTLD-ASSOCIATED SQTSM1 MUTATIONS EXERT THEIR EFFECTS IN A DOMAIN-DEPENDENT MANNER WHICH MAY CONVERGE ON AUTOPHAGY

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Keywords: Autophagy, SQSTM, LC3

Background: Autophagy is a critical pathway for the degradation of damaged and aggregation-prone proteins and organelles. Dysregulation has been proposed as a pathophysiological mechanism in ALS/FTLD. The p62/SQSTM1 protein is a multi-domain protein with a key role in autophagy, which mediates a critical protein-protein interaction with ATG8/LC3 proteins within the autophagosome membrane (*via* its LC3-interacting region, LIR) to transport ubiquitinated cargo to the developing autophagosome. Mutations affecting the SQSTM1 gene, which encodes the p62/SQSTM1 protein, have been identified in patients with ALS/FTLD and the majority map to specific functional domains within p62/SQSTM1 including the LIR.

Objectives: To determine whether ALS/FTLD-mutant p62/SQSTM1 proteins exhibit functional defects relevant to autophagy.

Methods: The effects of several ALS/FTLD-associated mutations of p62/SQSTM1 (within different domains) on protein function were analysed using a combination of biochemical (fluorescent microscopy, protein pull-down and co-immunoprecipitation assays, and NF-κB reporter assays) and biophysical techniques (isothermal titration calorimetry (ITC) and nuclear magnetic resonance (NMR) spectroscopy).

Results: *In vitro* pull-down assays showed that UBA domain mutations (T430P and G425R) impacted p62/SQSTM1's ability to bind to ubiquitin whilst mutations in other domains retained ubiquitin-binding. Only UBA domain mutations (T430P and G425R) were associated with a 5-fold increase in NF-κB activity relative to wild-type (wt)-p62/SQSTM1 ($n=3$ $p<0.0001$) in reporter assays.

Fluorescence microscopy of NSC-34 cells transfected with p62/SQSTM1 revealed the T430P mutation had a more diffuse localisation than wt-p62/SQSTM1 and did not aggregate as readily, whereas all the other mutants tested had similar expression phenotypes to wt-p62/SQSTM1.

A Keap1-interacting region (KIR) mutant P348L was the only mutation to selectively impair the p62/SQSTM1-Keap1 interaction in co-immunoprecipitation assays, whilst the LIR mutant L341V selectively reduced p62/SQSTM1's affinity for LC3 in *in vitro* pull-downs.

More detailed analyses with a peptide representing the LIR showed the L341V mutant had an approximately 3-fold weaker binding affinity for LC3 compared to wt-p62/SQSTM1 in ITC binding experiments (K_d 3.2 ± 1.1 μM compared to 10.9 ± 1.1 μM). NMR titration experiments revealed the L341V mutant's structural effects are localised around the LIR binding site on LC3.

Discussion and conclusions: The decreased binding affinity of the L341V LIR mutant to LC3 supports the

idea that dysregulation of autophagy is important in the aetiology of ALS/FTLD associated with SQSTM1 mutations. Although the difference in affinity is subtle, it may become biologically important over time in motor neurones. Collectively these results point to multiple distinct disease mechanisms but due to complex cross talk between the signalling pathways they may functionally converge on autophagy. Restoring autophagic activity in affected neurons could be an attractive therapeutic approach to fight ALS/FTLD.

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P229 HUMAN SPINAL VENTRAL HORN CONTAINS A LOWER AUTOPHAGIC CAPACITY THAN OTHER CNS AREAS: IMPLICATION FOR SELECTIVE VULNERABILITY OF MOTOR SYSTEM IN ALS

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Keywords: autophagy, spinal motor system, selective vulnerability

Background: A central unanswered question in ALS research is why the spinal motor system is selectively vulnerable to mutations in the ubiquitously expressed, aggregation-prone, superoxide dismutase-1 (SOD1). We have previously found that $SOD1^{G127insTGGG}$ (G127X) aggregates are enriched in spinal ventral horn, but not in other regions of the central nervous system (CNS), from ALS patients. Since protein aggregates are degraded by the autophagy pathway, we hypothesize that the low capacity of autophagy in spinal motor system may contribute to accumulation of SOD1 aggregates, leading to selective vulnerability of the system.

Objectives: The aim of the present study was to compare autophagic capacity in multiple areas of the CNS in human ALS patients.

Methods: A total of 28 human subjects was examined: Five non-neurological controls, five Parkinson's disease (PD), four sporadic ALS, five familial ALS without *SOD1* mutations, and nine familial ALS with *SOD1* mutations including A4V, G72C, D90A, and G127X. Specimens of CNS gray matter, including temporal lobe, frontal lobe, cerebellar vermis, spinal dorsal horn, and the lamina IX of the spinal ventral horn were obtained at autopsy. Autophagic capacity in the CNS were evaluated using Western blot with several autophagic factors: Beclin 1, LC3-II and p62, which are a principal initiator, an inducible marker, and a dysfunctional marker of autophagy, respectively. To address whether the low capacity of autophagy is associated with loss of motor neurons, we counted the alpha-motor neurons, immunostained with a neuronal marker NeuN, in the ventral horn of spinal cords from $SOD1^{G127X}$ mice with heterozygous deletion of the *Becn1*, at different stages of the disease.

Results: Beclin 1 level was significantly lower in the spinal ventral horns than in other areas of the CNS from non-neurological controls and PD patients. The levels of LC3-II and p62 were also low in the ventral horns of the control groups. In contrast, ventral horns from ALS patients exhibited elevated levels of Beclin 1, more so in carriers of *SOD1* mutations than in apparently sporadic patients and carriers of other ALS-linked mutations. However, these concentrations only approached those found in other areas of the CNS in the control groups. There were also increases in p62 but not in LC3-II. In other areas of the CNS from ALS patients, autophagic capacity was identical to that in controls. Heterozygous deletion of *Becn1* in $SOD1^{G127X}$ mice did not accelerate loss of the motor neurons at a presymptomatic stage. However, the low capacity of autophagy by the *Becn1* deletion significantly exacerbates loss of the motor neurons by 55% and 31% at a symptomatic and terminal stage, respectively.

Conclusion: Low capacity of autophagy may be related to selective vulnerability of spinal motor system in ALS.

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P230 DISEASE-PROMOTING IMMUNOLOGICAL ALTERATIONS IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: G-CSF, biomarkers

Background: Amyotrophic Lateral Sclerosis (ALS) also known as Lou Gehrig's disease, represents a progressive and fatal neurodegenerative disorder affecting motor neurons with a typical median disease course of 2 to 5 years and a lifetime risk of 1:400 (1). Despite the expanding research interest, the heterogenous etiology of ALS in combination with the lack of validated biomarkers and active therapeutic agents obstruct effective treatment of this orphan disease. In the last decades a growing number of studies including cell culture experiments, animal models, but also human studies indicate immune and inflammatory abnormalities to contribute to the pathogenesis of ALS. (2). It has been demonstrated that during disease progression a protective Type 2 (pre-symptomatic or stable disease) traverses into a neurotoxic Type 1 immune response (progressive disease) (3).

Methods: Here we first reveal the activation state of the TGF-β pathway, including the most important downstream molecules within spinal cord (SC) and motor cortex (MC) tissue homogenates (kindly provided by Prof. Thal, MND network Germany) of ALS and control patients via Western Blot analysis and *in situ* hybridisation. Next, we investigated the neurogenesis in SC and MC tissue of ALS and controls by measuring the expression profiles of early neuronal differentiation and proliferation markers. Subsequently, we examined the SC and MC tissue for a correlation of tissue-infiltrating immune cells and local neuronal, glial, and microglial components. Finally, we determined the hematological alterations of plasma samples obtained from ALS patients and healthy controls regarding their levels of leukocytes, chemokines, pro- and anti-inflammatory cytokines, and vascular as well as angiogenic factors using electrochemoluminescence and FACS analysis.

Results: We were able to demonstrate enhanced circulating levels of pro-inflammatory cytokines (IFNγ, TNFα, IP-10) within the serum of ALS patients compared to healthy controls. Further, a trend towards reduced expression of Tie-2 and VEGF-C indicated diminished angiogenesis in ALS patients. Finally, there was a positive correlation between the circulating levels of monocytes and the ALS-FRS-R and an inverse correlation of mobilized eosonophils and the ALS-FRS-R with the latter being confirmed by enhanced levels of plasma eotaxin of ALS patients compared to healthy controls.

Discussion and conclusion: Taken together, the results of the current study might shed some light on biomarker discovery and provide possible targets for a successful treatment development in ALS.

Acknowledgements: Spinal cord and motor cortex tissue was kindly provided by MND Network Germany.

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P231 AGE-RELATED CHANGES IN THE INFLAMMATORY CYTOKINE ENVIRONMENT SURROUNDING FACIAL MOTONEURONES

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Keywords: cytokines, ageing, motor neurones

Background: We previously reported that advancing age affects facial motor neuronal survival in the rat brainstem[(1)]. Recently, increased inflammatory cytokines in both the blood and the central nervous system (CNS) have been put forward to explain aspects of ageing, such as neurodegeneration and decreased motor function[(2)]. However, these views are based on studies of single inflammatory markers and do not distinguish changes in the CNS from those occurring in the blood.

Objectives: To compare age-related changes in (i) multiple cytokines in the rat brainstem with those occurring in the blood (systemically); (ii) general exploratory behaviour.

Methods: The behaviour of groups of 3-5 female Sprague-Dawley rats aged 3, 12-18 and 24 months was observed in an open-field test[(3)]. Rats were then deeply anaesthetised, blood plasma retrieved and stored at −80°C. After transcardial saline-perfusion, the brainstem was homogenised and the supernatant stored at −80°C. After protein estimation assays, IL-1α; IL-β; IL-2; IL-4; IL-5; IL-6; IL-10; IL-12p70; IL-13; TNFα; IFN-γ and GM-CSF were assayed using multiplex technology with a Magpix Luminex instrument[(4)]. A general linear model generated descriptive statistics and the multivariate ANOVA and Dunnett's post-hoc tests reported statistical significance of $p<0.05$ (*) and $p<0.01$ (**).

Results: 3m old rats had higher mean concentrations of IL-5, IL-6 and IFNγ compared to 12-18m rats (*) in the brainstem, with no significant differences in the plasma. However, 3m old rats had lower mean concentrations of IL-1α (*), IL-2 (**), IL-4 (**), IL-10 (*) and TNFα (**) compared to 24m old rats in the brainstem. Interestingly, 3m old rats had higher mean concentrations of IL-12p70 and TNFα (*) in plasma compared to 24m old rats. In addition, 24m old rats had higher levels of IL-1α (*), IL-4 (**), IL-6 (**), IL-13(*), TNF-α (**), IFNγ (*) and GM-CSF (*) in the brainstem compared to 12-18m old rats with no differences in plasma. Both 3m- and 12-18m old rats showed larger social-exploratory behaviour compared to 24m old rats (*). In a separate preliminary study of 3m rats (n=4), 0.1mg/kg intraperitoneal lipopolysaccharide (LPS), largely recreated the cytokine changes in seen in aged rats.

Discussion and conclusions: Contrary to current assumptions, our results indicate that ageing in rats is associated with a general decline in peripheral inflammatory cytokines and a general increase in inflammatory cytokines in the CNS. Recreation of the cytokine changes with LPS suggests a primary involvement of the innate immune system.

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P232 NAV3, AN AXONAL GUIDANCE PROTEIN ABERRANTLY EXPRESSED IN ALS

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Keywords: Nav3, axonal guidance, motor neuron

Background: Various axonal guidance proteins have been implicated in ALS including semaphorins, ephrins and Nogo-A (1). However, it is still unknown whether alterations in levels of these proteins is a contributing cause or a consequence of the denervation of neuromuscular junctions in ALS. Neuron navigator 3 (Nav3) is a microtubule-binding axonal guidance protein mainly expressed in the nervous system (2). Recently, Nav3 RNA but not protein was found to be decreased in frontal cortex of Alzheimer's patients, and Nav3 RNA was determined to be regulated by miR29Am (3). In an unbiased proteomic analysis of CSF from ALS patients, our group found Nav3 protein to be significantly increased in ALS compared to controls. These findings were further corroborated by a transcriptional increase in Nav3 in ALS lumbar motor neurons(4).

Objective: To investigate the role of Nav3 in ALS using *in vivo* and *in vitro* models of disease, and human tissue samples.

Methods: CSF and lumbar spinal cord protein homogenates were used to validate changes in Nav3 levels in ALS compared to controls by western blot. Immunohistochemistry was performed on hippocampus and lumbar spinal cord sections to compare Nav3 cellular expression and protein localization. *In vitro* models consisting of primary rat motor neurons and SH-SY5Y neuroblastoma cells were used to further investigate mechanistic effects of Nav3 overexpression on cellular viability, morphology, and microtubule-based protein trafficking.

Results: Nav3 protein levels were increased in SALS lumbar spinal cord homogenates compared to controls, and western blot analysis from ALS CSF samples similarly showed increases in the levels of this protein. In the SOD1G93A ALS animal model, increases in Nav3 RNA started at the symptomatic day 90 stages, although they failed to reach significance, while robust significant increases appeared at the post-symptomatic 120 day stage. *In vitro* mechanistic analysis of Nav3 revealed this axonal guidance protein to be dramatically upregulated transcriptionally in response to oxidative stressors Paraquat and H_2O_2. Using a fluorescent Nav3 fusion protein, we followed the movement of Nav3 in neuronal processes *in vitro* and determined movement aberrations in response to stress.

Discussion and conclusions: Taken together, we describe a novel axonal guidance protein altered in ALS, and are investigating its mode of action and/or potential contribution to disease pathobiology.

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Theme 12 *In vitro* Experimental Models

P233 A NEW PROTOCOL FOR GENERATING FUNCTIONAL MOTOR NEURONS FROM HUMAN STEM CELLS

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Keywords: motor neurons, human, stem cells

Background: The motor neuron (MN) is the central focus of a group of diseases, typically motoneuron diseases (MND) such as amyotrophic lateral sclerosis (ALS), a fatal disorder of MN degeneration. Our previous study presented the *in vitro* generation of functional MNs from human spinal cord stem cells (1).

Objectives: In this study, we aim to develop a new protocol that can generate functional hMNs from human stem cells with improved efficiency and the induced MNs can be maintained with better long-term stability in culture.

Methods: cell culture, immunostaining, electrophysiology

Results: As an alternative to sonic hedgehog (SHH), the key ventralization factor in MN induction during development, Purmorphamine and smoothened agonist (SAG) were investigated in their efficiency in MN induction. SAG generated the highest percentage of MNs based on Islet1 immunostaining. SAG was then introduced to the ventralization step during the procedure. Since the induced culture is a mixture of a variety of different cell types, and the presence of some populations of cells were noticed to disturb the stability of the culture and cause cell detachment after 10 days, we developed an opti-prep protocol to remove the un-desired cell populations. The generated culture can then be maintained over 2 months. The electrophysiological properties of these differentiated MNs were monitored during the culture period to confirm their functional integrity. The majority of these MNs fired repetitive action potentials (APs), which is an indicator of functional maturation.

Discussion and conclusions: This study developed a new protocol that can generate functional human MNs with improved efficiency and the MN cultures can be kept for long-term stability. The functional human MN culture that has long stability will provide a powerful *in vitro* model to study human MNs in terms of their physiology, insult-induced pathology and therapy, for both acute and chronic situations.

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P234 BANKING AND DISTRIBUTION OF MOTOR NEURONS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS: A FOCUS ON MOTOR NEURON DISEASES

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Keywords: disease modeling, iPS cells, motor neurons

There are many challenges involved in the production of valuable, consistent cell products that may be used to model diseases across different laboratories. We have addressed some of these challenges by creating a human induced pluripotent stem cell (iPSC)-derived motor neuron production core for disease modeling studies and translational work. In particular, we are focusing our efforts on producing banks of motor neurons derived from patients with motor neuron diseases such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) using cutting-edge differentiation techniques for derivation and banking. Here we describe our banking and quality control methodology. Key aspects to the process include: standard operation procedure development and implementation, barcoded sample and metadata tracking throughout the manufacturing process using custom inventory software, and rigorous purity, potency and identity assays. Alongside these differentiation protocol optimizations, we are also developing genome-wide methods to assay global mRNA and protein expression in these cells. These analyses aim to sensitively gauge technical variability across batches, assess similarities to *in vivo* motor neurons, and detect biologically meaningful differences between disease and control conditions. Rigorous accounting of all these factors will be crucial for effective motor neuron disease modeling.

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P235 C9ORF72 IPSC-DERIVED MOTOR NEURONS AND CORTICAL NEURONS SHOW FEATURES OF ALS AND FTD

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Keywords: C9orf72, iPS cells, mitochondria

Background: An expanded hexanucleotide (GGGGCC) in repeat in chromosome 9 open reading frame 72 (C9orf72) is a major cause of familial amyotrophic lateral sclerosis (fALS) and frontotemporal dementia (FTD). The function of the C9orf72 gene and its pathogenic mechanisms are currently unknown.

Methods: To investigate the phenotypes associated with the C9orf72 mutation in motor (MNs) and cortical neurons (CNs), we used induced pluripotent stem cells (iPSCs) derived from the skin fibroblasts of three patients carrying between 500 and 1000 G4C2 repeats in the C9orf72 gene. Pluripotency was induced by Sendai viruses carrying Sox2, Oct3/4, Klf4 and c-myc and 8 iPSC lines were used for characterization. Motor neurons were differentiated using three different protocols optimized in our laboratory. Cortical neurons were differentiated according to a widely used protocol. Functionality was assessed by electrophysiology, live calcium imaging and electron microscopy.

Results: We have followed-up our previous findings of altered ER Ca^{2+} homeostasis by assessing the contribution of mitochondrial dysfunction to neuronal degeneration in both MNs and CNs from C9 patients. In parallel studies, differentiating MNs and CNs from the same patients, we identified reduced mitochondrial potential in both MNs and CNs from ALS/FTD patients, which correlated with increased levels of pro-apoptotic Bcl-2 family members (BAX and BAK) and reduced anti-apoptotic members (Bcl-2 and Bcl-XL) in MNs. Furthermore, we detected increased levels of cytochrome c in the C9 patient lines compared to controls, which correlated with the activation of caspase-dependent apoptosis. While the presence of dipeptides was not confirmed in our cellular models, p62 and PABP aggregates were detected in up to 50% of C9 MNs and CNs.

Conclusions: Our cellular model of C9orf72 iPSC-derived motor neurons reveals disease-specific Ca^{2+} dysregulation which associates with ER stress and mitochondrial dysfunction, as well as the activation of apoptosis. The C9orf72 iPSC-derived motor and cortical neurons will be a valuable tool for investigating the mechanistic relevance of these phenotypes to disease and to develop efficient therapies.

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P236 GENERATION AND MOTOR NEURON DIFFERENTIATION OF INTEGRATION-FREE INDUCED PLURIPOTENT STEM CELLS (IPSCS) FROM ALS PATIENTS HARBORING FUS MUTATIONS

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Keywords: FUS, iPSC, motor neuron

Background: Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disorder due to selective loss of motor neurons. In 10% of patients, ALS is a familial disease. One of the genetic causes are mutations in the FUS gene. How these mutations result in the disease is still unknown.

Objective: To model FUS-ALS in a dish, we generated integration-free induced pluripotent stem cells (iPSCs) from ALS patients with different FUS mutations by using Sendai virus reprogramming. Meanwhile, we differentiated these iPSC into motor neurons and did initial phenotype characterizations

Methods: Fibroblasts from ALS patients with FUS R521H and FUS P525L point mutations and their unaffected family numbers were infected with Sendai-virus containing to reprogramming kit from Invitrogen. Immunostaining, quantitative RT-PCR, teratoma formation analysis and integration assays were used to identify the pluripotency of and absence of Sendai viruses in these iPSCs. Furthermore, motor neurons were derived from the iPSCs by inhibiting the Smad pathway and by activating the Hedgehog signaling (Shh) pathway. Initial gene expression assays of motor neurons were done by using quantitative RT-PCR and immunostaining.

Results and discussion: Three iPSC lines carrying a FUS R521H point mutation from 3 different patients, 3 iPSC lines carrying a FUS P525L point mutation from 1 patient and 2 iPSC lines from 2 different unaffected family members of the patient were established. Preliminary data indicate that motor neurons derived from ALS patient showed a decreased mRNA expression level of NF-L and NF-M, with an increased expression of NF-H. Both Na^+ and K^+ channel markers decreased in mutant motor neurons. In addition, FUS mislocalization was also observed in patient derived neurons. These results indicate FUS proteinopathy, imbalance of neurofilaments and hypoexcitability in FUS-ALS patients. Future work will

focus on further testing the initial results in more iPSC lines by using more technologies.

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P237 DIRECT CONVERSION OF ALS PATIENT FIBROBLASTS HARBORING FUS MUTATIONS TO INDUCED NEURONS DEMONSTRATES FUS ABNORMALITIES IN ALS NEURONS

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Keywords: fused in sarcoma, iPS cells, stress granules

Background: Fused in sarcoma (FUS) is a nuclear protein with a prion-like domain, RNA-recognition motif, and a nuclear localization signal (NLS). The function of FUS requires its proper localization in the nucleus. The mislocalization of FUS in the cytoplasm results in its loss of function in the nucleus and cytoplasmic inclusions of FUS in many neurons, which is sufficient to cause motor neuron degeneration.

Objectives and methods: To gain insight to FUS pathology in ALS, we designed 7 FUS variants associated with Korean ALS patients (X527YextX, Q519E, G504WfsX12, R495X, G399V, S235G, and 228_230delGGG), six of which we previously reported and one was newly identified. We performed functional analysis using murine neurons and human cell lines overexpressing FUS mutants, and patient fibroblasts carrying mutant FUS genes. In order to develop an accurate disease model, we converted the patient fibroblasts to induced neurons (iNeurons) by repressing a single RNA binding polypyrimidine-tract-binding (PTB) protein (1, 2).

Results: Overexpression of mutant FUS in human cell lines and murine neurons led to both nucleus and cytosolic distribution of G504WfsX12 and R495X, and their assembly into cytoplasmic stress granule (SGs) under oxidative stress. Contrary to our expectations, ALS patient fibroblasts carrying mutant FUS (G504WfsX12, R495X) revealed more abundant endogenous FUS immunoreactivities in the nucleus than the cytoplasm. On the other hand, the patient iNeurons with G504WfsX12 and R495X exhibited more abundant endogenous FUS immunoreactivities in the cytoplasm than the nucleus. Intriguingly, the patient iNeurons with Q519E mutation recapitulated the FUS pathology similar to iNeurons with G504WfsX12 and R495X, whereas, in the fibroblast model, Q519E was similar to healthy controls.

Discussion and conclusions: FUS (G504WfsX12) is pathogenic as it showed a similar pattern of subcellular distribution to R495X in both fibroblast and iNeuron models. Cytosolic distribution of FUS (Q519E) in the patient iNeurons compared to its nuclear distribution in fibroblasts demonstrates that iNeuron model is more functionally relevant with ALS-associated FUS mutations than the conventional *in vitro* cell models.

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P238 TDP-43 MISLOCALISATION IN PRIMARY MOTOR AND CORTICAL NEURONS FROM A NATIVE-PROMOTOR-DRIVEN TDP-43 ALS MOUSE MODEL AS A HIGH CONTENT AUTOMATED IMAGE BASED SCREENING PLATFORM

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

Background: TAR DNA-binding protein 43 (TDP-43) is characteristically depleted from the nucleus of affected neurons in ALS, where it forms the major protein component of insoluble, ubiquitinated inclusions in the cytoplasm. We have previously developed a novel bacterial artificial chromosome (BAC) transgenic mouse that expresses human TDP-43 at physiologically-relevant levels. TDP-43^{M337V} mutant mice develop a distinct motor and pathological phenotype recapitulating the key features of human ALS.

Objective: Full characterisation of the *in vitro* phenotype of motor and cortical neurons from TDP-43^{M337} transgenic mice to optimise TDP-43 mislocalisation as a readout for high content automated image based screening.

Methods: To explore the function of mutant TDP-43, we generated primary motor neurons from E13.5 lumbar

spinal cord from non-transgenic (NTg), TDP-43^{WT} and TDP-43^{M337V} heterozygous embryos by P75 immunopanning. Cortical neurons were generated from the cortex of new-born (P0) mice. TDP-43 was detected using an antibody with equal affinity for mouse and human forms of the protein. Human-specific TDP-43 and GFP (Ypet) antibodies were used to detect the human-specific form. To examine the effect of oxidative stress, MNs were treated with 0.05M sodium arsenite for 0, 30 and 60 min prior to fixation. All MNs were analysed at 7 or 10DIV.

Results: TDP-43^{M337V}-derived primary motor and cortical neurons demonstrate the characteristic cytoplasmic mislocalisation of TDP-43 under basal culture conditions. A significant proportion of mutant-derived primary neurons display cytoplasmic mislocalisation of TDP-43 compared to NTg and TDP-43^{WT} controls, and overall there is a shift to cytoplasmic localisation of the protein. Preliminary data suggests the TDP-43^{WT} vs TDP-43^{M337V} cytoplasmic distribution of TDP-43 staining is robust (Z Factor[1] for the cytoplasmic localization = 0.65 (n=3)). Pilot experiments to determine the dynamic range and standard deviation of the results are underway.

Discussion and conclusions: In collaboration with the Oxford Target Discovery Institute we will develop an automated computerised imaging system to establish high-throughput screening (HTS) of FDA approved compounds and a 'druggable' siRNA library. We aim to use HTS to identify drugs and drug targets which promote nuclear retention of TDP-43, which can then be validated through detailed analysis of phenotypic and transcriptional changes in primary neurons from our TDP-43 BAC transgenic mice, and iPSC-derived motor and cortical neurons from ALS patients carrying TDP-43 mutations.

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P239 MODEL OF TDP-43-MEDIATED CELLULAR TOXICITY IN HUMAN IPSC-DERIVED NEURONS

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Keywords: TDP-43, iPSC-neurons, AAV

Background: TAR DNA-binding protein 43 (TDP-43) was identified as the cardinal protein in the most common subtypes of frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). TDP-43 is a widely expressed nuclear protein that binds both DNA and RNA. While shuttling between nucleus and cytoplasm, it helps regulate many aspects of RNA processing, such as splicing, trafficking, and stabilization. In neurodegenerative diseases, neuronal TDP-43 becomes mislocalized to the cytoplasm, where it aggregates into stress granules and insoluble inclusion bodies. These inclusions occur in 97% of patients with ALS and tau-negative FTD.

Objectives: The goal of our study was to develop a cellular model that recapitulates the phenotype of TDP-43 mislocalization as seen in ALS and FTD in human induced-pluripotent stem cell (iPSC)-derived neurons (hu-neurons).

Methods: We compared the effects of adeno-associated virus (AAV8)-induced expression of Flag-tagged full-length wild type TDP-43 and TDP-43 carrying ALS-associated mutations, A90V or A315T in hu-neurons. Functional characterization of the AAV8-infected hu-neurons was carried out for the formation of TDP-43 cytoplasmic inclusions, stress granules, changes in acetylated tubulin and neuronal viability using high content image (HCI) analyses and AlphALISA.

Results: Evaluation of flag stained hu-neurons by HCI showed ~50% of neurons infected with either WT or A90V mutant. Both endogenous and overexpressed TDP-43 was mostly localized to the nucleus, but cytoplasmic mislocalization of TDP-43 was found in about 8-10% of hu-neurons infected with either WT or A90V mutant as early as 3 days post AAV infection. Time course evaluation showed a progressive increase of TDP-43 cytoplasmic inclusions at 7 and 14 days post AAV- infection of both WT and A90V and a parallel reduction in cell viability. Levels of acetyl-tubulin were also significantly reduced with overexpression of either TDP-43 WT or A90V mutant at both 7 and 14 days. At 7 days post-AAV infection, treatment with thapsigargin, an ER stressor, resulted in an increase in expression of TIA-1, stress granule markers and cellular toxicity. The effects are more pronounced in neurons infected with A90V mutant TDP-43 compared to WT TDP-43.

Discussion and conclusion: Our work provides validation of a novel cell model of TDP-43 pathology in hu-neurons to enable mechanistic understanding of TDP-43 associated proteinopathies. Efforts are ongoing to further evaluate relevant biological mechanisms that are involved in TDP-43-mediated cellular toxicity, effect on synaptic plasticity and identify key targets/pathways of interest for therapeutic intervention.

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P240 A NEW CELLULAR MODEL OF PATHOLOGICAL TDP-43: THE NEUROTOXICITY OF STABLY EXPRESSED CTF25 OF TDP-43 DEPENDS ON THE PROTEASOME

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Keywords: TDP-43, the CTF25 of TDP-43, oxidative stress

Background: The C-terminal fragment-25 (CTF25) of TDP-43 is a fragment of TAR DNA-binding protein 43 kDa (TDP-43),which is involved in RNA metabolism, neurite outgrowth, neuronal development and stress granules. Not until recently did evidence suggest that CTF25 might play an important role in amyotrophic lateral sclerosis (ALS) pathogenesis (1-3). However,

mechanistic details on CTF25 causing motor neuron degeneration still remain unknown.

Objectives and methods: To study the toxicity of CTF25 of TDP-43, we established a cellular models stably expressing CTF25 of TDP-43.

Results: Herein, we found that stably expressed CTF25 could induce significant oxidative stress and was mainly degraded by the proteasome pathway in cells. Furthermore, the neurotoxicity of CTF25 of TDP-43 was dependent on proteasome activity. In addition, electron microscopy showed mitochondrial swelling and cristae dilation in cells expressing CTF25 and that CTF25 aggregates were characterized by filamentous bundles and electron dense granular material.

Discussion and conclusions: The new cellular model mimics classical toxic TDP-43 cellular model and interestingly the toxicity of CTF25 is dependent on the proteasome.

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P241 FIBROBLASTS FROM PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS) ASSOCIATED WITH MUTATIONS IN TARDBP GENE AS MODEL OF TDP-43 PROTEINOPATHY

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Keywords: Stress granules, fibroblasts, mutant TDP-43

Background: *TARDBP* encoded protein, TDP-43, has been implicated in both the sporadic (sALS) and familial (fALS) ALS cases and has multitude of RNA and DNA regulatory functions. Animal models of TDP-43 have been inconclusive and contradictory and the role of TDP-43 in ALS remains an enigma to date. Fibroblasts obtained from the patients carrying mutations in TARDBP gene provide a vital tool in investigation of TDP-43 due to physiological levels of TDP-43. Formation of stress granules (SG) requires perfect synchrony of RNA binding proteins and is formed in a protective response to exogenous stress. Previous studies on over-expression TDP-43 models have focussed on localisation of TDP-43 into SG. We present the results of impaired SG dynamics in three different mutant TDP-43 patient derived cell lines expressing physiological levels of mutant TDP-43.

Methods: Immunocytochemistry was performed on three lines of control and three different TDP-43 mutant fibroblast lines (M337V, G287V, A321V) and confocal microscopy was performed to identify general TDP-43 and phosphorylated TDP-43 localisation. Anti p62 was used to identify ubiquitinated aggregations. Fibroblasts were also subjected to 0.5mM arsenite, 2μM thapsigargin, 0.4M sorbitol or a 42°C heat shock, and the stress response was assessed using markers of stress granules such as TIAR and HUR. Recovery after stress was also assessed.

Results and conclusion: In keeping with the findings in *post mortem* material from ALS cases, relative clearing of nuclear TDP-43 was noted in mutant TDP-43 fibroblasts ($p<0.001$). TDP-43 fibroblasts also showed accumulation of p62 positive aggregates ($p<0.0003$), and phosphorylated TDP-43 accumulation ($p<0.001$) compared to controls, suggesting that mutant TDP-43 fibroblasts share some characteristics of the surviving motor neurons from both sALS and fALS. Following exogenic stress endogenous TDP-43 localised to HUR positive stress granules ($p<0.01$), however assembly and disassembly of stress granules were less robust in mTDP-43 cases compared to that of controls ($p<0.01$) suggesting that dysfunctional TDP-43 dysregulates handling of exogenic stress. We suggest that impaired exogenous stress handling may contribute to the premature degeneration of motor neurons expressing mutant TDP-43 in ALS patients. Fibroblasts also form a robust platform to study TDP-43 related neurodegeneration.

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P242 DISEASE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS/ PARKINSONISM-DEMENTIA COMPLEX IN THE KII PENINSULA

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Keywords: Kii ALS/PDC, iPSC, disease model

Amyotrophic lateral sclerosis (ALS) and parkinsonism-dementia complex (ALS/PDC) in Kii peninsula, Japan is characterized by: 1) accumulation of the patients in the specific local regions; 2) dense familial history; 3) that some patients have parkinsonism, dementia and amyotrophy; 4) diffuse neurofibrillary tangles lesions in central nervous system; and 5) existing only in Japan and Guam island. Kii ALS/PDC pathogenesis remains unclear, and there are neither effective animal models nor therapeutic approaches. In order to determine the cellular consequences of the disease, we used somatic cell reprogramming to generate patient-specific induced pluripotent stem cells (iPSCs) by transfecting the episomal plasmids into peripheral blood mononuclear cells and T lymphocytes of patients. We confirmed that these established cells showed a typical morphology of human pluripotent stem cells, normal karyotypes, the expression of pluripotency markers and no episomal vectors remained. We successfully differentiated these iPSCs into neurospheres, and furthermore induced into dopaminergic neurons, motor neurons and astrocytes. We investigated disease-specific phenotypes in them, as a result, revealed the accumulation of phosphorylated tau and increased oxidative stress in the neuronal cells derived from Kii ALS/PDC patients-iPSCs. The neuronal cells derived from the iPSCs we established is a novel and excellent disease model of Kii ALS/PDC.

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P243 AN *IN VITRO* MODEL FOR TBK1 HAPLOINSUFFICIENCY IN PRIMARY NEURONAL CULTURES

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Keywords: TBK1, autophagy, primary neuronal cultures

Heterozygous loss-of-function mutations in TANK-binding kinase 1 (TBK1) cause ALS. TBK1 is a pleiotropic kinase that is involved in both inflammatory pathway signaling and regulation of autophagy. It remains to be shown whether dysregulated neuro-inflammation, impaired protein homeostasis or both are the relevant downstream effects mediating motoneuron degeneration in TBK1 mutation carriers. In order to establish a neuronal *in vitro* model for TBK1 haploinsufficiency, we generated lentiviral vectors expressing shRNA against TBK1 mRNA. Using different shRNAs, we achieved various degrees of reduction in TBK1 protein expression in cultured mouse primary neurons. An approximately 50% reduction in TBK1 protein levels led to an impairment of autophagy in neurons, indicated by accumulation of p62 and LC3 protein levels. Furthermore, already this mild TBK1 dosage reduction resulted in reduced viability and induction of caspase activity, while expression of a scrambled control shRNA had no effect on autophagy or survival. Our data indicate that the TBK1 haploinsufficiency, as well as its detrimental effects on neuronal maintenance as it is found in ALS patients with TBK1 loss-of-function mutations, can be modelled in cultured mouse neurons. Moreover, our findings point to an at least partially motoneuron-autonomous induction of neurodegeneration by TBK1 dosage reduction. We will also present data elucidating relevant downstream events, specifically with regard to a disturbed protein quality control and turnover of ALS-related aggregating proteins.

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P244 SMALL MOLECULE BASED HIGH-EFFICIENCY DIFFERENTIATION OF MOTOR NEURONS FROM IPS CELLS DERIVED FROM CONTROL AND FALS PATIENT FIBROBLASTS

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Keywords: iPS cells, differentiation, Islet1

Background: Inducible pluripotent stem cells (iPSC) have become an important model of familial motor neuron disease, which allows the study of human motor neurons *in vitro*. However, differentiation is prolonged and yields are variable, especially if the differentiation requires the generation of embryoid bodies.

Objective: To establish a small molecule based monolayer motor neuron differentiation protocol that can be used to efficiently generate large quantities of spinal motor neurons in a non-automated tissue culture laboratory.

Methods: iPS cells from 2 controls and 2 C9orf72 expansion carriers were cultured in confluent wells of a 6-well plate on Matrigel. We incorporated the results from a recent systematic combinatorial analysis of motor neuron differentiation (2) into an existing monolayer differentiation protocol (1). In brief, iPSCs grown on Matrigel were induced using Compound C and Chir99021. On day 3, 1mM retinoic acid and 0.5mM SAG were added. On day 9 cells were passaged onto Geltrex and the appearance of OLIG2 positive cells was observed until the majority of cells became positive for this marker. After day 16 DAPT and neural growth factors were added. The identity of the cells was confirmed via immunofluorescence microscopy for Islet1, SMI-32 and ChAT.

Results: We successfully differentiated iPS motor neurons from patient and control cell lines using the above protocol. Olig2 positivity increased from 17% on day 10 to 90% on day 16. At 24 days cells had a monomorphic neuronal appearance, remained stable in culture and were 92% positive for SMI-32 and 94% positive for ChAT. There were no differences between cases and controls. Interestingly, antibodies against Islet1, an embryonal transcription factor frequently used to stain motor neurons, showed much lower positivity, ranging between 11-45% of all cells, even in SMI-32/ChAT positive cells.

The physiological properties of the motor neurons were confirmed by calcium signalling.

Conclusion: Our method generated a high-yield motor neuron culture from iPSCs within three weeks of neural induction. We achieved this using commercially available small chemicals on 6-well tissue culture plates without the need for embryoid bodies or specialised equipment. In line with others we found that Islet1 was only transiently expressed, requiring the use of alternative motor neuron markers to assess the differentiation efficiency.

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P245 IMMORTALIZED HUMAN NEURONAL PROGENITOR CELL LINE (RENCELL CX) IS AN IDEAL MODEL TO INVESTIGATE REGENERATION OF ADULT NEUROGENESIS IN THE SVZ

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Keywords: Adult Neurogenesis, Regeneration, TGF-beta

Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative lethal disorder with no effective treatment so far. The current molecular genetic campaign is increasingly elucidating the molecular pathogenesis of this fatal disease; from previous studies it is known that Transforming Growth Factor-β (TGF-β) is found in high concentrations in Cerebrospinal Fluid (CSF) (1) of ALS patients. These high levels of circulating TGF-β are known to promote stem cell quiescence and therefore lead to inhibition of adult neurogenesis within the sub ventricular zone (SVZ) of the brain (2). Thus, regeneration of degenerating neurons seems to be prevented by an enhanced TGF-β signaling.

Objectives: In this study we wanted to figure out if selective inhibition of TGF-β signaling mediated by a selective TGF-βRII-antisense-oligonucleotide might allow regeneration of adult neurogenesis. Therefore, we investigated if an immortalized human neuronal progenitor cell line isolated from the cortical region of human fetal brain (ReNcell CX; Millipore) is a proper model to analyze the TGF-β-mediated effects.

Methods: First, we examined the ability of ReNcell CX cells to respond generally to TGF-β by analyzing the respective molecules up- and downstream of the canonical TGF-β pathway. Further, ReNcell CX cells were treated with 10 or 50 ng/ml TGF-β1 for 7 d to initiate stem cell quiescence. ReNcell CX cells were also exposed to different concentrations (2.5 and 10 μM) of a TGF-βRII-antisense-oligonucleotide by gymnotic delivery to examine subsequently the consequence of TGF-βRII downregulation on Smad-cascade and stem cell markers as well as TGF-β mediated inhibition of stem cell quiescence. All probes were analyzed by quantitative RT-PCR, immunoblotting and immunocytochemistry.

Results: So far, we were able to show that ReNcell CX cells react on TGF-β1 treatment with a significant increase of its signaling (pSmad2), corresponding elevation of its response genes (CTGF, PAI-1) and even self-induction of TGF-β1 has been recognized. Furthermore, we could show that TGF-β1 treatment leads to cell cycle arrest by downregulation of proliferation markers (Ki67, PCNA). Also, the ReNcell CX cell line demonstrated a proper downregulation of TGF-βRII and showed thereby an inhibition of TGF-β signaling and a corresponding upregulation of stem cell markers like Nestin and Sox2 due to gymnotic transfer of a selective TGF-βRII-antisense-oligonucleotide.

Disscusion and conclusions: Results indicate that ReNcell CX cells represent an adequate model to investigate regeneration of adult neurogenesis by selective antisense oligonucleotide-mediated blocking of TGF-β signaling due to TGF-β response, cell cycle arrest following TGF-β–exposure and upregulation of stem cell markers after TGF-βRII downregulation. Thus, new therapeutic strategies addressing neurogenesis can be analyzed easily with ReNcell CX cell line.

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P246 DEVELOPMENT OF PERSONALIZED *IN VITRO* NEUROMUSCULAR SYSTEM FOR ALS/MND STUDIES AND DRUG SCREENING

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Keywords: NMJ, iPS cells, personalized medicine

Background: One of the most prominent features of ALS is the heterogeneity not only in its triggering mechanisms but also in its disease paradigm. While no particular cause has been specified for spontaneous ALS (sALS, 90% of

the cases), over 30 genes have been identified to be involved in familial ALS (fALS) (1) and each gene appears to target a different subcellular mechanism (1, 2). Correspondently, multiple therapeutic strategies have been proposed targeting different cell types and mechanisms, unfortunately with little success due to poor efficacy and limited generality (1, 3). Therefore, it is essential to develop accurate models for ALS, which can be tailored to each particular case, or on a personalized model. Additionally, although ALS is accepted as a motoneuron (MN) disease, some evidence suggests that neuromuscular junction (NMJ) pathology represents a more sensitive and crucial landmark for the progression of the disease.

Objectives: This study aims to develop personalized functional NMJ system for ALS etiological study and drug screening, with human MNs (hMNs) derived from ALS patients.

Methods: cell culture, immunostaining, electrophysiology

Results: A human-based functional *in vitro* NMJ system has been developed by utilizing human stem cells as the source (4). To tailor it into specific ALS cases, hMNs with genetic mutations of SOD1, FUS, C9orf72 etc were first differentiated from iPSCs derived from ALS patients. The morphology and functionality of the hMNs were then characterized by immunocytochemistry and electrophysiology. They were then co-cultured with human skeletal myotubes (hSKM) (5) in a medium that encourages NMJ formation. The formation of functional NMJ will be analyzed by immunostaining and Glutamate/Curare assay. The quantity of functional NMJ can be monitored until complete loss. The dynamic changes of the function and viability of hMNs in the co-culture can also be monitored by electrophysiology and immunocytochemistry.

Discussion and conclusions: This personalized *in vitro* functional NMJ system represents a more accurate model for ALS, due to its utilization of patient-derived cells and its focus on NMJ integrity, an early clinic hallmark for disease onset (6).

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P247 FUNCTIONAL INTERROGATION OF ACTIVE NEUROMUSCULAR SYNAPSES BETWEEN HUMAN STEM CELL-DERIVED MOTONEURONS AND HUMAN SKELETAL MUSCLE *IN VITRO* BY A MULTI-ELECTRODE CANTILEVER ARRAY

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Keywords: functional, cantilever, iPS cells

Background: High-content screening of drugs aimed at mitigating the adverse effects of motoneuron diseases (MNDs) necessitates the establishment of physiologically accurate *in vitro* models. Since MN death is not observed in disease models of ALS, the focus of pharmacological interventions has switched to reversal of functional and/or morphological degeneration at the neuromuscular junction (NMJ) (1). Past work in our lab has demonstrated the capacity for human cells to form NMJs *in vitro*, through immunocytochemistry and electrophysiology (2,3). In this work we obtain functional output of the NMJs using a specialized co-culture system.

Objective: To develop a platform that allows the interrogation of NMJ functionality and perform a basic demonstration where MN stimulated muscle contraction is attenuated by the addition of drugs that block nicotinic acetylcholine receptors found in the synaptic cleft of NMJs.

Methods: Human MNs and skeletal muscle are plated on opposite sides of a PDMS barrier with MNs on microelectrodes and muscle on cantilevers. Microtunnels guide the MNs' axons through the barrier to the muscle where they synapse. The barrier provides electrical and chemical isolation leaving the axons as the only significant conduit for communication between the two cell types. Motoneuron stimulation causes action potentials to propagate down their axons triggering NMJ mediated contractions of the muscle. Conversely, broad field stimulation on the muscle side of the barrier causes muscle contractions through direct depolarization. The muscle contractions are monitored by a laser reflected off the cantilevers.

Results: Electrical coupling of the co-culture system is demonstrated by observing muscle contractions upon stimulation of either side of the barrier. Bungarotoxin, which inhibits the binding of acetylcholine to the nicotinic acetylcholine receptor at the NMJ, is then cumulatively added to the muscle side. The result is the eventual cessation of muscle contractions upon motoneuron side stimulation while no such effect is observed by muscle side stimulation. The progressive decline in functionality after each dose is observed as a decrease in contractile strength and the muscle's ability to pace with the stimulation.

Discussion and conclusion: The ability to quantify the extent of functional decline upon the addition of neuromuscular blockers is a sensitive screening system which,

when used in reverse, could aid in the discovery of novel drugs that improve functionality. Continuing work involves adapting this system to study MNs differentiated from IPSC derived from patients with ALS to test drugs that can lead to a potential cure for this neurodegenerative disease.

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P248 NSC-34 MOTOR NEURON-LIKE CELLS COMPARED TO MOTOR NEURONS TO INVESTIGATE GLUTAMATE-MEDIATED EXCITOTOXICITY

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Keywords: in vitro models, NSC-34 cell line, glutamate-mediated excitotoxicity

Background: The glutamate induced-excitotoxicity mechanism is a major contributor to motor neuron degeneration in the pathogenesis of amyotrophic lateral sclerosis (ALS). NSC-34 cell line is often used as a *bona fide* cellular model to investigate physiopathological mechanisms of ALS. However, the physiological response of NSC-34 to glutamate remains insufficiently described.

Objectives: In this study, we propose to evaluate the relevance of differentiated NSC-34 ($NSC\text{-}34_D$) as an *in vitro* model for glutamate excitotoxicity studies. Expression of glutamate receptors in several differentiation conditions as well as glutamate-induced excitotoxicity and calcium (Ca^{2+}) transients were studied.

Methods: NSC-34 cells were differentiated by serum depletion with or without *all-trans* retinoic acid (RA), the expression of genes encoding glutamate receptor subunits and certain specific motor neuron proteins were evaluated by RT-PCR and compared to primary motor neurons. To assess the effect of glutamate-induced excitotoxicity in $NSC\text{-}34_D$, cells were exposed to L-glutamic acid at different concentrations for 48 h before determination of cell viability by trypan blue assay. To address the effects of glutamate on Ca^{2+} transients in cells we used ratiometric Ca^{2+} measurement with the imaging dye Fura-2AM

Results: $NSC\text{-}34_D$ showed several morphological and physiological motor neuron-like properties and expressed glutamate receptor subunits *GluR1-4*, *NR1* and *NR2A/D*. Despite this evidence of expression, we did not observe any specific effect of glutamate on $NSC\text{-}34_D$ survival, although it has been reported in primary motor neurons. Moreover, we did not observe any significant increase in cytosolic calcium concentration following exposure of $NSC\text{-}34_D$ to glutamate compared to primary motor neurons

Discussion and conclusion: Our findings suggest that the motor neuron-like NSC-34 cell line is not a suitable *in vitro* model for glutamate excitotoxicity studies in the context of ALS or any motor neuron disorder involving this pathophysiological mechanism. We recommend the use of primary motor neurons to explore pathogenesis of glutamate-mediated excitotoxicity

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P249 MODULATING THE MCU/VDAC WITHIN THE ERMCC IN ALS

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Keywords: MCU, VDAC, ERMCC

Background: Amyotrophic lateral sclerosis (ALS) is a severe neurodegenerative disease. In ALS the ER-mitochondria-calcium-cycle (ERMCC) is disrupted characterized by Ca^{2+} depletion of the ER and Ca^{2+} overload of the mitochondria. Ca^{2+} crosses the outer mitochondrial membrane via the voltage dependent anion channel (VDAC) that is also involved in the formation of the mitochondrial permeability transition pore (mPTP) in extreme stress situations. The main regulator of the mPTP is Cyclophilin D that acts under physiological conditions as peptidyl-prolyl-isomerase. Furthermore, Ca^{2+} is channelled through the inner mitochondrial membrane by the mitochondrial calcium uniporter (MCU) complex which consists of the pore forming MCU and regulatory proteins and is driven by the electrochemical gradient.

Objectives: (i) to elucidate the VDAC, Cyclophilin D and MCU expression in primary motor neuron cell cultures from hSOD1^{G93A} mice compared to wild type mice (ii) to determine the effect of modulation of these proteins on the ERMCC function.

Methods: Motor neurons were isolated from embryonal spinal cords and seeded on primary astrocyte cultures, both from hSOD1^{G93A} and wild type mice. After two weeks, cells were harvested and qRT-PCR was performed to evaluate mRNA expression levels. Furthermore, two weeks old cells were fixed with 4% PFA and stained using immunofluorescence against MCU, VDAC and Cylophilin D. To determine the effect of modulators on the proteins, before fixation or harvesting, Ruthenium Red was added to the media and cells were incubated for 24 hours.

Results: Immunofluorescence staining showed the presence of all three investigated proteins in the soma and axons of hSOD1^{G93A} and wild type motor neurons. Quantitative analysis of immunofluorescence showed significantly higher levels of MCU in hSOD1^{G93A} motor neurons than in wild type ($p=0.02$). Results for VDAC and Cyclophilin will be provided. qRT-PCR will give further information about expression levels of MCU, VDAC and Cyclophilin D in hSOD1^{G93A} and wild type motor neurons and non-motor neurons.

Conclusions: Up-regulation of MCU and VDAC is a possible reason for Ca^{2+} overload of mitochondria in ALS while an up-regulation of the MCU regulator MICU1 is a protective mechanism to prevent Ca^{2+}-induced apoptosis pathways during disease progression. Higher expression of VDAC and Cyclophilin D enhances the probability of the formation of the mPTP that leads to apoptosis in most cases and may represent the event of "no turning back".

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P250 ALS MODELS - THE CONTRIBUTION OF PATIENTS' LYMPHOBLASTOID CELL CULTURES

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Keywords: lymphoblastoid cell culture; SOD1, TARDBP, FUS mutations

Background: In ALS, a main difficulty is the identification of a valid pathological model. Since clinically ALS affects MNs, they would be the best candidates. Unfortunately patient's MNs are available only *post-mortem* and give little information on early disease stages. Cellular and animal models are also useful, however translation from animal to patients is laborious and not completely reliable. Regarding patient's peripheral tissues, we reported that PBMCs from sALS patients display signs of oxidative stress (SOD1 and Bcl-2 alterations) reflecting MNs modifications (1; 2).

Objectives: We verified if the molecular signatures could be associated with gene mutation (SOD1, TARDBP, FUS) in PBMCs from ALS patients. Considering that PBMCs cannot be grown and stored as a cell line and do not show the handling flexibility needed for "modeling", we switched our attention to lymphoblasts, EBV-immortalized lymphocytes, to verify if PBMCs molecular signatures were maintained.

Methods: SOD1, TARDBP and FUS transcript levels were analyzed in PBMCs from mutated, sALS patients and controls. SOD1 mRNA was evaluated in lymphoblasts from SOD1mut patients to verify PBMCs result reproducibility. In lymphoblasts, we investigate SOD1, TDP43 and FUS cellular localization by WB and immunofluorescence. Apoptosis and mitochondrial dynamics were analyzed by WB. Mitochondrial morphology was studied by TEM.

Results: PBMCs from SOD1mut patients showed SOD1 mRNA altered levels, the mutated allele was up-regulated. In lymphoblasts SOD1 mRNA levels increased. In total soluble fraction, SOD1 protein levels were reduced and immunofluorescence showed SOD1 recruitment in cytoplasmic aggregates. No changes in Cyt-C release were observed; a slight increase in DRP1 levels indicated that fission is favored. TEM evidenced smaller mitochondria with disorganized cristae and vacuoles.

TARDBPmut patients showed increased levels of TARDBP transcripts. Lymphoblasts cytoplasmic extracts presented increased levels of full-length TDP43 protein. Immunofluorescence showed cytoplasm with round-shaped TDP43-containing aggregates. An increased cytoplasmic Cyt-C release suggested an apoptotic status and elevated MFN1 levels promoted the fusion pathway. Mitochondria presented giant masses containing electron dense globes and vacuoles.

In FUSmut patients, mRNA levels did not change. In lymphoblasts no cytoplasmic mislocalization was observed. Cytoplasm Cyt-C suggested apoptosis. MFN1 levels were increased and TEM reported giant and degenerated mitochondria.

Discussion and conclusions: Patient-derived lymphoblasts display features typical of degenerating MNs: impaired RNA metabolism, protein aggregation and mitochondrial dysfunction. Lymphoblasts are intriguing as an ALS cellular model to study specific pathological pathways or identify new ones. Since a patient's blood is relatively easy to obtain and lymphoblastoid culture can simply be established, it is possible to collect mutation-specific samples, allowing a comparison between pathological signatures of different mutations, leading to patient stratification on a molecular basis.

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Theme 13 *In vivo* Experimental Models

P251 ZEBRAFISH C9ORF72 LOSS OF FUNCTION MODELS OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: C9ORF72, zebrafish, loss of function

Background: A GGGGCC repeat expansion in chromosome 9 open reading frame 72 (*C9orf72*) is a major cause of both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), together referred to as C9FTD/ALS. It is unknown how the repeat expansion causes C9FTD/ALS, however there is evidence for three possible, non-exclusive pathogenic mechanisms. Reduced levels of C9orf72 mRNA in patients has been reported, suggesting C9orf72 loss-of-function via haploinsuffiency may be causative in C9FTD/ALS. Alternatively, pathogenic mechanisms may involve a toxic gain-of-function of the repeat containing transcripts via RNA toxicity and/or repeat-associated non-ATG (RAN) translation into aggregation-prone, dipeptide repeat (DPR) proteins (C9RAN proteins). *C9orf72* codes for 2 protein isoforms of unknown function, however there is emerging evidence that it may play a role in protein trafficking, as a member of the DENN-like superfamily.

Objectives: To generate a stable zebrafish *C9orf72* loss-of-function model using targeted genome-editing techniques. 1) To use this model to investigate if C9orf72 loss-of-function via haploinsuffiency causes C9FTD/ALS; 2) To investigate the function of C9orf72 *in vivo* in a vertebrate model.

Methods: The CRISPR/Cas9 system and transcription activator-like effector nucleases (TALENs) genome editing techniques were utilised to target sequences within exon 1 and 7 of *C13H9orf72,* the zebrafish orthologue of *C9orf72,* respectively. To investigate the effects of C9orf72 loss-of-function we are currently measuring survival, motor function and behaviour in stable zebrafish mutant lines.

Results: In total, four TALEN lines carrying nonsense mutations within exon 7 of *C13H9orf72* have been investigated. These mutations are predicted to result in a truncated form of C13H9orf72. As there is not a reliable antibody to detect C13H9orf72 protein levels in the zebrafish, qRT-PCR was used to investigate whether these truncated C13H9orf72 transcripts are degraded by nonsense-mediated decay. In comparison to wild type lines, each of the homozygous mutant lines show a reduction in *C13H9orf72* mRNA levels of approximately 60-80%. The mutations in *C13H9orf72* did not lead to early loss in viability. Motor function and behaviour are being characterised. CRISPR/Cas9 targeted against exon 1 of *C13H9orf72* successfully transmits a variety of insertion and/or deletion (INDEL) mutations through the germline and work is in progress to raise additional stable mutant lines generated using this technique.

Conclusion: We have generated stable zebrafish lines carrying loss-of-function mutations in *C13H9orf72*. Characterisation of these zebrafish is in progress, but initial results using embryos suggests there is no early effect on the motor system or early loss of viability.

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P252 IDENTIFICATION OF A NOVEL NEUROPROTECTIVE DRUG FOR ALS USING "ZNSTRESS", A ZEBRAFISH HIGH THROUGHPUT PHENOTYPIC SCREEN AND VALIDATION IN THE SOD1 G93A MOUSE MODEL

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Keywords: phenotypic screen, drug discovery, zebrafish

Background: Mutations in the SOD1 gene are one of the major causes of familial ALS. SOD1 misfolding is also strongly implicated in sporadic ALS; thus drugs that impact SOD1 mediated pathogenesis have huge potential in treating ALS. Mutant SOD1 zebrafish developed in our lab show all the hallmark features of ALS (1). Phenotypic screens have re-emerged as an important methodology in drug discovery.

Objectives: Here we used a transgenic sod1 zebrafish model of ALS to screen for neuroprotective compounds by utilizing a novel fluorescence reporter that labels stressed neurons in the mutant sod1 transgenic zebrafish (2). Cells respond to the presence of misfolded proteins by the induction of a heatshock stress response (HSR), which in our transgenic fish produces a quantifiable red fluorescence signal. We hypothesized that drugs that impact SOD1 misfolding in vulnerable cell groups would inhibit the HSR, thus reducing neuronal stress. We then tested the efficacy of a lead compound from the zebrafish screen in the SOD1 G93A mouse model of ALS.

Methods: 48hpf SOD1 transgenic zebrafish were treated by immersion with compounds obtained through the "Microsource spectrum library" until 6 days post fertilisation (dpf). At 6dpf the larvae were sonicated and total fluorescence measured using a fluorescence plate reader.

Strictly standardised mean difference (SSMD) was used to select hit compounds (β-value below -0.5 were considered as hits in this assay). Hits from this screen were tested for dose response and a lead compound was selected for further testing for efficacy in the C57Bl6 SOD1G93A mouse model of ALS.

Results: A high-throughput drug screen has been developed with the capability to screen 1000's of compounds with an assay specificity and sensitivity approaching 100%. We have screened 2000 compounds from the Spectrum library and identified 61 different modulators of neuronal stress. From this screen we have identified 42/2000 compounds which reduced the neuronal stress signal and 19/2000 which induced an increased hsp70 activation. Using secondary screens we have identified a selection of hit compounds that have a positive role in reducing neuronal stress in mutant sod1 zebrafish. Dosing of the lead compound to wild-type mice showed that the drug is safe and can reach a level in the CNS predicted to be sufficient for neuroprotective efficacy. The lead compound is currently being tested in the mutant sod1 G93A mouse model.

Conclusions: Use of a mutant SOD1 zebrafish model of ALS is suitable for high throughput drug screening and serves as an excellent *in vivo* platform for bringing promising lead compounds for testing in pre-clinical mouse models of ALS.

Acknowledgements: This work is supported by the MND Association.

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P253 DIFFERENTIAL TOXICITY OF NUCLEAR RNA FOCI VERSUS CYTOPLASMIC LOCALIZATION OF FTD/ALS-ASSOCIATED GGGGCC REPEATS IN DROSOPHILA

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Keywords: C9ORF72, drosophila, iPSC

A noncoding GGGGCC (G_4C_2) repeat expansion is the most common genetic mutation in both ALS and FTD. Some dipeptide repeat (DPR) proteins produced through repeat-associated non-ATG (RAN) translation are toxic in cellular and animal models. However, it is unclear whether nuclear RNA foci formed by expanded G_4C_2 repeats also induce neurotoxicity. Here, we describe a novel *Drosophila* model expressing up to 160 G_4C_2 repeats flanked by human intronic and adjacent exon sequences. Intronic 160 G_4C_2 repeats, efficiently spliced out as in human cells and patient brains, formed nuclear G_4C_2 sense RNA foci in neurons and glial cells about 10 times more abundantly than in human neurons; however, these foci had little effect on RNA processing and neuronal survival. In contrast, 36 G_4C_2 repeats expressed in the context of poly(A)$^+$ mRNA were exported to the cytoplasm and highly toxic with DPR proteins produced at >150-fold higher level than by intronic 160 repeats. Thus, nuclear RNA foci may be neuroprotective by sequestering expanded G_4C_2 repeats, resulting in a reduced production of toxic DPR proteins.

Acknowledgments: We thank F. Ladam, E. Ricci, Z. Zhang, S. Ordway, and Gao lab members for discussions and comments; the Bloomington *Drosophila* Stock Center for fly lines; and the Department of Cell and Developmental Biology Confocal Core at the University of Massachusetts Medical School for help with some images. We also thank L. Ranum for anti-GR antibody, E. Baehrecke for anti-p62 antibody, and W. Seeley (Neurodegenerative Disease Brain Bank at the University of California, San Francisco) for brain tissues of *C9ORF72* patients. This work was supported by an ALS Association Milton Safenowitz Postdoctoral Fellowship (H.T.), a grant from ALS Therapy Alliance (F.B.G), a grant from the Department of Defense (ALSRP AL130125 (F.B.G. and L.P.)), Mayo Clinic Foundation (L.P.), and grants from the ALS Association, Packard Center for ALS Research, Target ALS (F.B.G. and L.P.). This work was also supported by grants from the National Institutes of Health: R01NS057553, R01NS079725 and R21NS086318 (F.-B.G.); P50AG016574, R21NS084528, R01NS088689, R01NS063964; R01NS077402 and P01NS084974 (LP); R21NS089979 (T.F.G.).

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P254 GENERATION AND PHENOTYPING OF A NOVEL MOUSE MODEL OF ALS-FUS USING BACTERIAL ARTIFICIAL CHROMOSOME (BAC) TECHNOLOGY

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Keywords: FUS, mouse model, phenotyping

Background: Mutations in the gene encoding the RNA-binding protein FUS (Fused in sarcoma) cause a subtype of ALS characterised by cytoplasmic mislocalisation of FUS, the extent of which correlates with disease onset and severity. A number of rodent models have been created in an attempt to recapitulate key features of ALS-FUS. The majority of these have been generated using a cDNA transgene and it is uncertain whether these models truly reflect the human disease, or instead demonstrate toxicity from FUS overexpression. Here we sought to generate a novel FUS mouse model, relevant to human disease, using the entire human FUS genomic locus together with BAC technology to express the human FUS transgene from the native promoter, avoiding overexpression.

Objectives: To create and phenotype a mouse model of ALS-FUS by expressing FUS from the entire human FUS gene using BAC transgenesis.

Methods: BAC constructs containing the entire human FUS locus were generated harbouring either the human wild-type (WT) FUS sequence or the ALS-associated P525L mutation. The FUS gene, including its introns, downstream of its own promoter and regulatory sequences, was cloned into a BAC and a sequence encoding an N-terminal mCherry fluorescent tag was fused to Exon 1. Founder mice were generated by pronuclear injection and independent mouse lines were established from each founder. Longitudinal behavioural testing was performed at 3 monthly intervals and body weight was monitored each month.

Results: FUS-BAC copy number, transgene mRNA and protein product levels have been characterised in experimental lines; revealing low-copy WT and P525L lines and a high-copy P525L line. Motor neurons cultured from the spinal cord of WT FUS-BAC mice display nuclear localisation of human WT FUS, while P525L FUS-BAC motor neurons demonstrate mislocalisation of mutant FUS to the cytoplasm. After oxidative stress P525L, but not WT FUS, accumulates in stress granules which are also reduced in number per cell. While there is no evidence of alterations in the distribution of endogenous SMN, EWS, or TAF15, P525L motor neurons display reduced survival *in vitro*. Additionally, longitudinal behavioural testing of locomotion, neuromuscular strength and cognition by open field, accelerating rotarod, 4-limb hanging wire test and spontaneous alternation over 12 months reveal subtle changes between lines.

Discussion and conclusions: Here we describe the generation of a novel FUS mouse model expressing WT or P525L mutant human FUS. We found altered stress granule dynamics in the presence of P525L FUS and reduced survival *in vitro*, which may reflect an underlying cellular vulnerability in the presence of this FUS mutation. Longitudinal behavioural testing suggests subtle transgene-related changes in the P525L lines; therefore this novel mouse model is ideally suited to explore early pre-symptomatic changes in ALS-FUS.

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P255 *IN VIVO* INVESTIGATION OF TRUNCATED FUS LOSS- OR GAIN-OF-FUNCTION

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Keywords: FUS, transgenic mice, RNAseq

Background: Mutations in the gene encoding Fused in Sarcoma (*FUS*) are the cause of 4% of fALS cases. Physiologically, FUS is a DNA/RNA binding protein which has been involved in several aspects of nucleic acid metabolism. It is highly debated whether mutations lead to loss of those functions or to the gain of new detrimental activities and how these might be linked to the pathogenesis of ALS.

Objectives: The aims of the project are 1) to investigate transcriptome changes secondary to Fus ablation in the developing mouse brain and 2) to assess whether human FUSwt or R495X associated with aggressive fALS can rescue those changes

Methods: *Fus*-/- mice were generated from stem cells carrying Fus targeted allele (EUCOMM International Knockout Mouse Consortium). RNA from brains collected at E18.5 was extracted with Trizol and used for deep-sequencing. Transgenic mouse lines expressing either HA-tagged human wild-type FUS (HA-hFUSwt) or the truncated protein FUS-R495X (HA-hFUSR495X) under the control of the prion protein promoter were generated by oocyte injection. Transgene distribution was assessed by immunohistochemistry and subcellular fractionation followed by western blot analysis. Cross-breeding between overexpressing and Fus-/- mice is ongoing.

Results: Brains from *Fus*-/- embryos display no residual Fus expression and were used for RNAseq followed by differential expression analysis thus revealing a broad impact on brain transcriptome with 1748 genes dysregulated (826 upregulated, 922 downregulated; adjp <0.05). Furthermore, 438 cassettes affecting 390 individual genes are differentially regulated by alternative splicing. Among these, only 83 are also differentially expressed, thus suggesting that Fus regulates gene expression through several mechanisms. In parallel, we characterized overexpressing mouse lines with different transgene expression. The transgenic line expressing highest amounts of hFUSR495X undergoes premature lethality. Interestingly, mouse lifespan is also slightly reduced in animals expressing moderate amounts of hFUSwt but not in those expressing comparable levels of hFUSR495X. Lacking the NLS, the truncated protein distributes predominantly in the cytoplasm, however, in the higher expressing line, robust nuclear transgene expression was achieved. Remarkably, lifespan reduction correlates best with nuclear, rather than cytoplasmic, overexpression.

Discussion and conclusions: Many mutations affect FUS subcellular localization thereby prompting to speculate that loss- or gain-of-function might arise from its cytoplasmic retention. We have now identified transcripts sensitive to Fus depletion thorugh exquisitively nuclear (i.e. alternative splicing) or cytoplasmic (i.e. altered mRNA stability) mechanisms. We have also characterized overexpressing mice with different expression of hFUSwt or hFUSR495X including a line with robust nuclear expression of hFUSR495X. Crossing *Fus*-/- mice with the overexpressing lines will allow us to investigate which transcription abnormalities can be rescued by hFUSR495X when its expression is predominantly cytoplasmic or when it can robustly detected in the nucleus as well, thereby providing insights into distinct mutant Fus functions.

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P256 A TDP-43Q331K TRANSGENIC MOUSE SHOWS PROMISE AS A ROBUST PRE-CLINICAL MODEL OF MOTOR NEURON DISEASE (MND)

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Keywords: TDP-43, transgenic mouse model

Background: Most current mouse models of MND are based on SOD1 mutations and are representative of only a small proportion of MND. Nearly all non-SOD1 patients have inclusions of the protein TDP-43, whereas SOD1 patients do not, suggesting a mechanistic difference between non-SOD1 and SOD1-related MND (1). Therefore, a mouse model based on mutations in TDP-43 may be more representative of sporadic disease with the potential to provide a more robust, translational model.

We are currently investigating two mouse lines which are transgenic for human TDP-43^{WT} or TDP-43^{Q331K} expressed at a similar level to endogenous mouse TDP-43, which demonstrate a mutant specific motor phenotype (2).

Objectives: To fully characterise the TDP-43^{Q331K} mouse model of MND using a variety of quantitative measures of disease progression, to enable the design of a statistically robust preclinical screening protocol.

Methods: Two mouse colonies were established, TDP-43^{WT} transgenic, and TDP-43^{Q331K} transgenic on the C57BL/6N background (Jackson Labs, 017907 and 017933 respectively) and bred as hemizygous lines. Both genotypes were investigated for phenotypic characteristics of MND using rotarod, automated gait analysis, weight measurements and running wheels.

Results: The TDP-43^{Q331K} mice showed signs of spasticity, tremor, muscle loss and weight gain. Male TDP-43^{Q331K} mice showed significantly worse rotarod performance from 9 weeks of age (39.4%-61.5% reduction between weeks 9 and 31, $P<0.05$, two way ANOVA, n=5-7/group); female TDP-43^{Q331K} mice from 12 weeks (30.5%-69.9% reduction between weeks 12 and 35, $P<0.05$, two way ANOVA, n=5-7/group), compared to all other groups. Automated gait analysis at 6 months of age indicated reduced limb swing speed and increased base of support in hind limbs (32.7±3.0mm in TDP-43^{Q331K} vs 25.5±2.2mm in TDP-43^{WT} mice, $p<0.001$, n=6-7) indicative of the spastic 'swimming gait' observed in these mice. Interestingly, TDP-43^{Q331K} transgenic mice showed significantly increased weight; in male TDP-43^{Q331K} mice from 11 weeks of age (18.5%-24.7% increase between weeks 11 and 31, $P<0.05$, n=5-7); female TDP-43^{Q331K} mice from 12 weeks (37.3%-40.4% increase between weeks 12 and 35, $P<0.05$, n=5-7). This increase in weight was observed despite a significant loss in triceps surae muscle mass observed at 6 and 10 months of age (198±26mg in TDP-43^{Q331K} vs 340±43mg in TDP-43^{WT} mice at 6 months, $p<0.001$, n=6-7).

Discussion and conclusions: TDP-43^{Q331K} mice show promise as a model of MND as they demonstrate a progressive motor degenerative phenotype. Spasticity, tremor, muscle loss and reduced motor performance support the validity of this model of MND, whereas the weight gain is unexpected. Further investigations are underway to assess the pathological phenotype of these mice.

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P257 ABSENCE OF WIDESPREAD MIS-SPLICING IN THE PRE-CLINICAL PHASE OF A NATIVE PROMOTOR-DRIVEN TDP-43 MOUSE MODEL OF ALS

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Keywords: RNAseq, Mouse, TDP-43

Background: TDP-43 pathology is the pathological hallmark of amyotrophic lateral sclerosis and rare mutations in its gene TARDBP have been linked to familial ALS. Using BAC transgenic technology we developed a TDP-43^{M337V} mutant mouse that expresses the transgene from a stable single copy, under the influence of the native human TDP-43 promotor. The TDP-43^{M337V} heterozygous mouse develops a progressive motor and pathological phenotype by 9-12 months of age.

Objective: To delineate longitudinal changes in mRNA expression levels and splicing in the spinal cord of a novel TDP-43^{M337V} ALS mouse model

Methods: Spinal cord was taken at 3, 9 and 12 months from four mice from each genotype: nontransgenic (NTg), transgenic human wild-type TDP-43 and transgenic human M337V mutant TDP-43. RNA was extracted using trizol reagent and the miRNEasy kit. RNA integrity was validated using Nanodrop analysis and the Agilent Bioanalyser. RIN scores for all extractions were above 8. Samples were prepared with the Illumina TruSeq RNA Sample Prep Kit v2. All sequencing was performed on the Illumina HiSeq HiSeq2000 platform using TruSeq v3 chemistry to a read depth of at least 26 million. All sequence was paired-end and performed over 100 cycles.

Results: For the 3-month time-point, sequencing data was found to be of good quality, with Phred scores consistently above 32 and read lengths of 100bp. There were 96% mapped reads across all mice and 75-78% of reads were expressed. 33 genes were significantly upregulated and 54 genes were downregulated in TDP-43^{M337V} compared to TDP-43^{WT}. Pathway level differential analysis revealed upregulation of genesets containing ribosomal proteins, translation elongation factors when comparing TDP-43^{M337V} to TDP-43^{WT}. No systemic splicing changes occur between TDP-43^{M337V} and TDP-43^{WT}. Further time-points are currently being sequenced.

Conclusion: In our mouse model of ALS, early expression changes, but no splicing changes are evident at 3 months of age. Further analysis of the RNAseq data at other time-points will enable us to observe changes and trends that develop as cellular pathology becomes apparent.

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P258 STAGES OF IMPAIRED EYE MOVEMENT CONTROL IN AMYOTROPHIC LATERAL SCLEROSIS ARE CONSISTENT WITH THE MODEL OF SEQUENTIAL SPREADING OF PTDP-43 PATHOLOGY

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Keywords: eye movement control, in vivo staging, video-oculography

Background: Eye movement investigations are a sensitive tool to gain deep insights into brain network pathology including 1) disturbance of the pulse generating part of the brainstem oculomotor circuitry as well as 2) executive deficits of oculomotor function associated with higher brain networks. We aimed to investigate whether eye movement disturbances in amyotrophic lateral sclerosis (ALS) allow a staging scheme of oculomotor pathology to be established that is consistent with the model of sequential corticofugal axonal spread of pTDP-43 pathology.

Methods: Eye movement recordings using video-oculography (EyeSeeCam) together with clinical (ALS-FRS) and neuropsychological scores (ECAS) from 68 ALS patients and 31 matched healthy controls were used for the analysis.

Results: Thirty patients presented no oculomotor deficits compared with controls. Eye movement abnormalities in ALS include a high distractibility as measured with anti- and delayed saccades errors and the occurrence of saccadic intrusions as well as 'genuine' oculomotor functions such as gaze-palsy or a cerebellar type of smooth pursuit disturbance. The sequential appearance of executive deficits and 'genuine' oculomotor abnormalities permit the categorization of eye movement pathology into two stages: Stage 1, only executive dysfunction, and stage 2, executive deficits plus impaired 'genuine' oculomotor functions such as gaze-palsy or impaired precerebellary circuits. The proposed oculomotor stages were significantly correlated ($p<0.001$) with both, the ALS-FRS, and the ECAS total score.

Conclusion: Executive deficits in eye movement control are the first manifestation, followed by impaired infratentorial oculomotor control pathways such as involved oculomotor nuclei and the brainstem circuitry for saccade generation. The suggested model of a two-staged sequential pattern of eye movement abnormalities in ALS is consistent with the neuropathological staging scheme and may serve as a technical marker of neuropathological progression in correlation to the clinical phenotype.

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P259 MUTANT PROFILIN1 TOXICITY IN THE TRANSGENIC MOUSE MODEL FOR ALS

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Keywords: Profilin1, motor neurons, ALS mouse model

The mechanism of neuronal degeneration and muscle atrophy in amyotrophic lateral sclerosis (ALS) is poorly understood. Current animal models have been helpful in defining disease development and identifying pathways and molecules potentially involved. This suggests more models are needed to increase our understanding of molecular mechanisms responsible for motor neuron death. Recent discoveries of new genes linked to ALS will

likely lead to new disease models and therapeutic targets. There are currently seven mutations in the profilin1 gene (PFN1) linked to fALS (1-3). This argues that mutation in PFN1 causes ALS in humans. However, the mechanism of mutant profilin1 toxicity and ALS pathogenesis remains unknown. To explore this relationship we have developed mouse models overexpressing mutant and wild-type (WT) human profilin1 (hPFN1) driven by mouse prion promoter. We chose the glycine to valine mutation at residue 118 (G118V) for our mutant hPFN1 mouse. These hPFN1G118V mice faithfully recapitulate ALS phenotype. We have now generated mice overexpressing WT human profilin1 (hPFN1WT). We found proteinopathy and multiple other abnormalities in our hPFN1G118V model while hPFN1WT mice are as viable and healthy as nontransgenic littermates. Here we report that lumbar spinal cords of symptomatic hPFN1G118V mice demonstrate a reduced F/G-actin ratio relative to WT mice, suggesting mutant profilin-1 may dysregulate actin polymerization. DNase I, which is another G-actin-binding protein and the most active apoptotic endonuclease, translocated to the nucleus in the hPFN1G118V mice. This DNase I redistribution in spinal cords correlated with the increase of nuclear DNA fragmentation measured using TUNEL assay indicating elevated cell death. We show mutant profilin-1 exists in the insoluble fractions of total spinal cord homogenates of PFN1G118V mice and most of proteins in the insoluble fraction are ubiquitinated. This confirms *in vitro* data published by Landers and colleagues and further suggests that aggregation of mutant profilin1 may contribute to neurotoxicity in ALS. In conclusion, we have developed two novel mouse models to investigate the role of mutant profilin1 in ALS. In characterizing these models we have found multiple ALS-like pathologies in the hPFN1G118V mice while hPFN1WT mice show no pathology as expected.

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P260 FURTHER CHARACTERIZATION OF MUTANT PROFILIN1 TRANSGENIC MOUSE MODEL FOR ALS

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Keywords: Profilin1, motor neurons, ALS mouse model

The mechanism of neuronal degeneration and muscle atrophy in amyotrophic lateral sclerosis (ALS) is poorly understood. Current animal models have been helpful in defining disease development and identifying pathways and molecules potentially involved. This suggests more models are needed to increase our understanding of molecular mechanisms responsible for motor neuron death and new models will be essential for preclinical therapeutic testing. There are currently seven mutations in the profilin1 gene (PFN1) linked to fALS (1-3). This argues that mutation in PFN1 causes ALS in humans. However, the mechanism of mutant profilin1 toxicity and ALS pathogenesis is unknown. To explore this relationship we have developed mouse models overexpressing mutant and wild-type (WT) human profilin1 (hPFN1) driven by mouse prion promoter.

We previously demonstrated that these mutant (hPFN1G118V) mice faithfully recapitulate ALS pathologies and phenotypes. PFN1 is indispensable for the formation of filamentous actin (F-actin) from globular actin (G-actin) and thus, cytoskeletal remodeling including axonal, dendritic outgrowth and transport of vesicles and mitochondria. PFN1 is therefore critical to motor neuron function and survival.

Our working hypothesis is that mutations in PFN1 impact actin polymerization leaving neurons with reduced F-actin, accumulation of G-actin, and aggregation of mutant profilin1. We will present data from lumbar spinal cord sections showing reduction of F-actin and increase of G-actin as measured by phalloidin and DNase I respectively, suggesting mutant profilin1 may disrupt actin cytoskeletal dynamics. We also show profilin1 exists in the insoluble fractions derived from total spinal cord homogenates of PFN1G118V mice as compared to hPFN1WT fractions. Insoluble fractions contain highly ubiquitinated protein species. Preliminary proteomic analysis from insoluble fractions confirmed the presence of human profilin1 protein by 9 folds in mutant samples and will be further analyzed. DNase I, another G-actin-binding protein and the most active apoptotic endonuclease, translocated to the nucleus in hPFN1G118V mice spinal cords. This DNase I redistribution correlated with the increase of nuclear DNA fragmentation measured using TUNEL assay indicating elevated cell death. We will present these new data resulted from further characterization of hPFN1 transgenic mice.

Support by grants from UAMS startup funds, the College of Medicine Research Council, Center for Translational Neuroscience, and NIGMS IDeA award P30 GM110702.

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P261 HETEROGENEITY OF MATRIN-3 EXPRESSION IN THE DEVELOPING AND AGING MURINE CENTRAL NERVOUS SYSTEM

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Keywords: Matrin 3, aging, development

Mutations in the *MATR3* gene encoding the nucleotide binding protein Matrin-3 have recently been identified as a cause for a subset of familial forms of amyotrophic lateral sclerosis (ALS) and were previously associated with distal myopathy. Translating the identification of *MATR3* mutations into an understanding of disease pathogenesis and the creation of a mouse model of these diseases are complicated by a paucity of data regarding Matrin-3 expression *in vivo.* Consequently, we examined the expression of murine Matrin-3 throughout different mouse tissues and regions of the central nervous system. We characterized Matrin-3 expression through post-natal development on whole brain and spinal cord tissue from naïve Swiss Webster mice at post-natal day one (P1), nine (P9), twenty one (P21), and thirty seven (P37). From P1 to P37, we observed a progressive reduction in levels of Matrin-3 (N = 3 per group. P1 v P21 and P1 v P37, $p<0.01$, one way ANOVA, Tukey *post hoc*). In adulthood, expression levels show regional variation within the CNS, and from cell to cell within the same tissue. Interestingly, the spinal cord was among the tissues with the lowest expression of Matrin-3. Furthermore, cells of the spinal cord normally show some cytoplasmic localization of Matrin-3. Lastly, we evaluated whether there may be changes in the expression or localization of Matrin-3 during aging in numerous CNS regions of three cohorts of C57BL/6 mice aged to one, four and twenty-one months of age. While Matrin-3 localization was unaltered with age, we observed significant reductions in the expression of Matrin-3 between the ages of one and four months of age in spinal cord, hippocampus, midbrain, and striatum (N = 4 per group. Spinal cord $p < 0.01$; hippocampus $p < 0.05$; midbrain $p < 0.05$; striatum $p < 0.001$, one way ANOVA, Tukey *post-hoc*). Our study is the first to characterize endogenous Matrin-3, providing the groundwork for future disease understanding and for the development of *in vivo* models of MATR3-linked distal myopathy and ALS.

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P262 ROLE OF CDNF IN SOD1-G93A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: SOD1, CDNF, ER-STRESS

Neurotrophic factors (NTFs) regulate the development, maintenance and plasticity of the nervous system (1) and in adult animals protect and repair injured neurons. Several NTFs promote the survival of motoneurons (MNs) *in vitro* and *in vivo*, thus being possible drug candidates for ALS. Among them, novel cerebral dopamine neurotrophic factor (CDNF) seems particularly promising, since it is highly expressed in muscle tissues, spreads better than other NTFs in the brain and rescues only degenerating neurons (2). Furthermore, CDNF is crucially involved in the regulation of the ER stress, which plays an important role in the pathophysiology of ALS. Here we show that intraventricular injection of human recombinant CDNF can significantly postpone appearance of clinical symptoms, improve motor coordination and increase lifespan in SOD1-G93A mouse model of ALS. CDNF treatment can prevent the death of MNs compared to controls and CDNF also preserves neuromuscular junctions (NMJs) in the studied gastrocnemius muscle. As mentioned above, ER stress is an important pathway to cell death in sporadic ALS patients and in ALS rodent models. We found the upregulation of the mRNA level of unfolded protein response (UPR) genes GRP78, Xbp1, PERK, ATF6 and CHOP in SOD1-G93A model, whereas their levels were reduced in CDNF-treated animals. Therefore our results strongly suggest that CDNF has a protective effect in SOD1-G93A mouse model of ALS, promoting the survival of MNs and the preservation of NMJs. The decrease in UPR genes mRNA level after CDNF treatment also suggests the intriguing possibility that CDNF might rescue MNs by regulating the ER stress response.

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P263 PROGRANULIN STIMULATES NERVE REGENERATION AND ENHANCES FUNCTIONAL RECOVERY *IN VIVO*

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Keywords: progranulin, frontotemporal dementia, nerve crush

Frontotemporal dementia (FTD) is a primary progressive, neurodegenerative disorder that is most common between the ages of 40 to 60 years and is clinically subdivided in one behavioral and two language variants. Approximately 40% of the patients have one or more affected relatives and in about 20% of these families loss of function mutations in the gene encoding progranulin (GRN) are causing the disease. A reduction of 50% or more in the levels of GRN in the blood and cerebrospinal is observed, suggesting haploinsufficiency of the GRN gene as pathogenic mechanism. *In vitro* experiments have already shown that GRN is a neurotrophic factor that stimulates nerve outgrowth and improves survival of primary neuronal cultures. To unravel the mechanisms by which GRN exerts its neurotrophic functions in the CNS, we used an *in vivo* paradigm of nerve regeneration. To this end, we generated GRN knockout mice and developed a nerve crush model to study axonal regeneration and functional recovery. A comparative study of the nerve crush model and a nerve axotomy model in the sciatic nerve showed equal neuromuscular junction denervation in the gastrocnemius muscle at 4 days post injury. Also, compound muscle action potential (CMAP) amplitude analysis and longitudinal sections of the sciatic nerve showed complete degeneration of the distal nerve segments after crush, similar to axotomy of the sciatic nerve. These observations were highly reproducible and showed low variability due to a homogeneous crush in all animals. To study the effect of GRN on functional recovery after crush, we subjected 8 to 12 week old GRN knockout mice and wild type littermates to a crush of the facial nerve. Whisker movement was scored daily and showed complete functional recovery by day 13 post crush in non-transgenic animals. In the absence of GRN, the overall functional recovery was delayed by one day and complete recovery was observed at day 16 post crush. Overexpression of hGRN could completely rescue this deficit. These observations suggest that GRN is an essential component in the nerve regenerative process and that hGRN can mediate these effects. Further research will be necessary to unravel the precise mechanism underlying this *in vivo* neurotrophic effect of GRN.

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P264 PROLONGED RELAXATION PHASE AS MEASURED BY TWITCH FORCE IN THE SOD1 G93A MOUSE MODEL

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Keywords: Muscle contraction, twitch force

Background: It is well known that weakness in amyotrophic lateral sclerosis (ALS) is direct result of motor neuron loss in brain and spinal cord. Less well appreciated is the fact that there are subsequent alterations in muscle mechanics that also impact strength. The contractile properties of muscle, as measured by twitch force, arise from kinetic processes within the myofilament system and the structural properties of the surrounding interstitium. Studies employing whole-muscle twitch measurements can help provide insights into potential changes in muscle mechanics that occur in ALS.

Objectives: The objective of this study was to evaluate twitch dynamics in ALS muscle of differing disease severity.

Methods: *In situ* twitch force experiments were performed on the gastrocnemius of 28 mice (B6SJL-Tg(SOD1-G93A)1Gur/J). Twitch force was recorded after stimulation by a single stimulus using 200 μs square pulse delivered to an insulated needle stimulating the sciatic nerve at the sciatic notch. Stimulation current and muscle length were adjusted to maximize twitch force. The twitch waveform was described by statistical moments analogously to a probability distribution. The shape of the twitches were characterized twitch maximum force, half-relaxation time, maximum rates of force rise and fall, contraction time, vertical and horizontal symmetry, mean time, standard deviation, skewness and excess kurtosis. Data were trichotomized according to age of the animals: mild (13 to 14 weeks, n=9), moderate (15 to 16 weeks, n=11), severe (17 to 18 weeks, n=8). Comparisons were performed with 1-way ANOVA (with post-hoc Tukey-Kramer).

Results: Maximal twitch force and speed of contraction were age-dependent ($p<0.001$). The contraction time did not differ between groups, due to a concurrent loss of force and speed of contraction. However, the half-relaxation time showed a trend toward increase between the mild and moderate groups ($p=0.08$) but did not extend to the severe group. This finding was supported by an increase in the horizontal symmetry among groups ($p<0.05$) caused by a widening of the twitch relaxation phase. Other measures were not found to be different between groups.

Discussion and conclusions: We have presented evidence that twitch dynamics are altered in ALS. We found that skeletal muscle has extended relaxation phase. This change may be due to nerve or muscle fiber hyperexcitability or the effects of non-functional elements within the muscle structure (e.g. denervated muscle fibers serving as an additional non-elastic element). We believe these early observations would benefit from further study, especially considering the potential for some of these alterations to be impacted by therapy with fast-troponin activators.

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P265 RESPIRATORY PLASTICITY IN THE SOD1(G93A) MOUSE MODEL OF ALS

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Keywords: respiration, circuit, plasticity

Respiratory failure is the leading cause of death in patients with Amyotrophic Lateral Sclerosis (ALS). Remarkably, both ALS patients and mouse models of the disease are able to maintain ventilation until late disease stages despite early and progressive degeneration of phrenic motoneurons innervating the diaphragm. However, the mechanisms of respiratory plasticity that maintain ventilation during periods of significant neurodegeneration are unknown. Equally elusive are those mechanisms that transition from respiratory compensation to ventilation failure, thus death, at disease endstage. Using a combination of unrestrained whole body plethysmography and continuous electromyography (EMG) to record from respiratory muscles we show a compensatory increase in the recruitment of accessory respiratory muscles early in disease progression in the $SOD1^{G93A}$ mouse model of ALS. During accessory respiratory muscle recruitment, we observe changes in both frequency and amplitude of inspiration, indicating that central respiratory rhythm generation is altered concurrently with increased muscle recruitment. Our model system will be used to develop and test potential therapies targeting respiratory circuits to improve breathing deficits in patients with sporadic or familial ALS.

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P266 EFFECT OF GENE COPY NUMBER ON DYSPHAGIA ONSET IN SOD1-G93A TRANSGENIC MICE

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Keywords: histology, SOD1-G93A, CGRP

Background: Nearly all people with amyotrophic lateral sclerosis (ALS) develop dysphagia (swallowing impairment) at some point in the disease progression. Dysphagia in ALS leads to malnutrition, dehydration, and pulmonary diseases that are highly correlated with a poor quality of life, morbidity, and mortality. The pathophysiologic mechanisms of dysphagia in ALS are largely unknown; therefore, targets for effective treatments remain elusive. The central theme of our lab is to identify mouse models of ALS suitable for investigating causative mechanisms and candidate treatments for dysphagia.

Objective: The goal of this study was to investigate the effect of gene copy number on the timing of dysphagia onset in two transgenic mouse models of ALS expressing SOD1-G93A: high copy number (HCN) and low copy number (LCN). These models were chosen because our previous work showed both models have clinical signs of dysphagia at disease end-stage.

Methods: Behavioral dysphagia assays were performed monthly on HCN, LCN, and age-matched littermate controls (>20 mice for each genotype, both sexes), beginning at weaning (21 days old). Assays included a lick rate assay developed in our lab. After testing, brains from 21 day old mice (HCN: n=4, LCN: n=4; controls: n=4) and tongues from 49 day old mice (HCN: n=13, LCN: n=14; controls: n=30) were collected for analysis. Brains were processed for brightfield immunohistochemistry (IHC) and transmission electron microscopy (TEM) of the hypoglossal nucleus. IHC analysis included density and morphometric measurements of several proteins (CGRP, SOD1, GFAP, IBA1). TEM analysis included form factor measurements (cell width:length ratio). Analysis of tongue samples included measurements of gross anatomy (area, length, width) and weight.

Results: Compared to controls, lick rate impairment was evident at 21 days of age for HCN mice and 6 months for LCN mice. IHC of the hypoglossal nucleus at 21 days revealed differences in only CGRP expression between genotypes: The proportion of high-density CGRP-staining motor neurons was significantly lower in HCN mice ($p<0.05$). The form factor of hypoglossal motor neurons was markedly variable for 21 day old HCN mice (0.245-1.293), compared to the uniform neuronal shape for age-matched LCN and control mice (0.500-0.734). No significant gross anatomical differences of the tongue were identified between genotypes at 49 days of age.

Conclusions: HCN mice have lick rate impairment at weaning (21 days), whereas impairment develops in LCN mice at 6 months. This novel, early clinical evidence of lingual dysfunction in HCN mice correlates with altered CGRP expression levels and form factor of hypoglossal motor neurons and provides molecular evidence of pathological neural development in this mouse model of ALS. These findings suggest LCN mice with adult-onset lingual dysfunction more closely recapitulate dysphagia onset in human ALS, and are therefore better suited than HCN mice for translational dysphagia research.

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P267 LOW COPY NUMBER SOD1-G93A MICE ARE BETTER SUITED FOR DYSPHAGIA RESEARCH COMPARED TO THE HIGH COPY NUMBER MODEL

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Keywords: dysphagia, videofluoroscopic swallow study (VFSS), swallow metrics

Background: Our lab investigates dysphagia in amyotrophic lateral sclerosis (ALS), predominantly utilizing the high copy number (HCN) expressing SOD1-G93A

transgenic mouse model. We have previously observed that these mice have dysphagia upon weaning, without other clinical signs of ALS. Therefore, we are investigating the low copy number (LCN) SOD1-G93A transgenic mouse model that has delayed onset of limb dysfunction and extended survival compared to HCN mice. Furthermore, LCN mice have forelimb and bulbar involvement that more closely resembles human ALS.

Objectives: The goal of this study was to use a videofluoroscopic swallow study (VFSS) protocol developed in our lab to characterize dysphagia in HCN and LCN mice at disease end-stage. Our ultimate goal is to identify a set of functional biomarkers that could facilitate early detection of dysphagia in ALS and serve as outcome measures to quantify treatment efficacy in clinical trials.

Methods: VFSS was performed on freely-behaving, disease end-stage LCN (n=13) and HCN (n=23) mice and age-matched nontransgenic littermate controls (n=23) of either sex. VFSS videos (recorded at 30 frames per second) were analyzed frame-by-frame to quantify 15 swallow metrics.

Results: Five of the 15 swallow metrics were significantly different ($p<0.05$) between all three groups (HCN, LCN, and controls): swallow rate, inter-swallow interval (ISI), pharyngeal transit time (PTT), lick rate, and bolus size. Specifically, SOD1 mice had slower swallow rates, longer ISIs, longer PTTs, slower lick rates, and smaller bolus sizes compared to controls. For all 5 metrics, HCN mice showed more severe swallow impairment compared to LCN mice.

Discussion: Both SOD1 mouse models of ALS (HCN and LCN) have significant clinical signs of dysphagia at disease end-stage; however the severity is more pronounced for HCN mice. Our previous finding that HCN mice develop dysphagia early in development (prior to weaning) provides strong rationale for preferentially including LCN instead of HCN mice in future investigations of dysphagia onset and progression in ALS. We are currently using this LCN mouse model for preclinical investigations to determine the optimal therapeutic window for preventing and/or slowing the progression of dysphagia in ALS, which may significantly improve life-span and quality of life. Furthermore, we have identified robust swallow metrics that may be adapted for use in human VFSS to facilitate early detection of dysphagia and quantify the effect of therapeutic interventions.

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P268 CHROMOSOME 17 MODIFIERS OF DISEASE PHENOTYPE IN THE G93A SOD1 AND DYCT1 MOUSE MODELS OF ALS

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Keywords: genetic modifiers, G93ASOD1, DYCT1

Background: We have previously demonstrated that the phenotype of both the G93ASOD1 transgenic mouse and the Dynactin p150Glued (DCTN1) mouse models of ALS are background dependent with a more severe phenotype (early onset and reduced survival) on the SJL or NOD background when compared to C57BL6 (B6). Furthermore, these variations in phenotype have been linked to a Chr17 quantitative trait locus (QTL) in the G93ASOD1 transgenic mouse. Using an interval specific B6 congenic mouse that carried a portion of SJL Chr17 genome from 17-52Mb, we demonstrated similar effects on phenotype in both the G93ASOD1 and the DCTN1 suggesting that at least some of the genetic modifiers are shared.

Objectives: To narrow the interval containing modifying genetic elements.

Methods: We developed interval specific congenic lines (ISC) on a homogeneous B6 (milder phenotype with late onset and prolonged survival) that contained portions of chromosome 17 (Chr17) derived from SJL (17-52 MB denoted B6.SJL17_17-52) or NOD (0-70 MB denoted B6.NOD17_0-70) strains. These ISCs have been bred and informative recombinants collected in order to develop ISC lines with smaller portions of the interval. To date we have derived a B6.SJL17_42-52, a B6.SJL17_35-42, and a B6.NOD17_0-24. The DCYN1 and G93ASOD1 transgenes have been bred onto each of these ISC mice and onset and survival collected.

Results: B6.NODChr17_0-70 mice expressing the G93AhSOD1 transgene demonstrated a shorter lifespan and earlier onset of motor neuron disease (MND). Both B6.SJL17_17-53 and 43-53 mice expressing the G93AhSOD1 transgene demonstrated early disease onset with no effect on survival. This data suggests that for the G93AhSOD1 mouse model, the survival modifiers map to the proximal 17 Mb whereas the onset modifiers are in the distal 43-52 Mb of the QTL interval.

Similar to G93AhSOD1 mice, the DYCT1 mice demonstrated delayed onset on B17.SJL17_17-53 ISC compared to the B6 background. However, survival in these animals not only was not shortened as expected with but was actually extended (538 days) when compared to transgenics on both SJL (275 days) and B6 backgrounds (444 days). This indicates that there may be an SJL derived modifier that prolongs survival in DYCT1 mice. Additional data is being collected for all of the ISC lines for both the G93ASOD1 and DYCT1 models and will be presented.

Conclusion: We have shown that background modifies phenotype in two different models of MND and identified a QTL in Chr17. We have also shown that some Chr17 modifiers are likely shared by these models. These shared modifiers would be of great importance in highlighting pathways of universal importance to motor neuron degeneration and provide targets for treatment and potential biomarkers of severity and disease prediction.

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Theme 14 Therapeutic Strategies

P269 EFFECTS OF HUMAN MESENCHYMAL STROMAL CELLS IN ALS *IN VITRO* AND *IN VIVO* MODELS

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Keywords: mesenchymal stromal cells, neuroprotection, growth factors

Background: Mesenchymal stromal cells (MSC) are potential candidates for the therapy of neurodegenerative diseases such as ALS and are already investigated in clinical trials, mainly based on the assumption that non-neuronal cells can provide a more protective environment for degenerating motor neurons.

Objectives: Having established a good manufacturing practice (GMP) conform protocol for the isolation of MSC from bone marrow available from healthy donors and appropriate quality control, we aimed to study effects of MSC in ALS *in vitro* models as well as in mutant SOD1 G93A mice and to define potential neurotrophic factors secreted by MSC.

Methods: Protective effects of MSC-coculture as well as MSC-conditioned medium against staurosporine toxicity were studied in motor neuron-like NSC-34 cells transfected with either plasmids containing mutant SOD1G93C and FUSR521C, respectively, fused to a sequence coding for eGFP or control plasmids only, as well in primary motor neurons from mutant SOD1-G93A ALS transgenic mice via immunocytochemistry and MTT assay. The impact of MSC on gene expression of growth factors and cytokines was studied by quantitative real-time PCR and the factors secreted by MSC were analyzed by Multiplex immunoassays.

SOD1-G93A transgenic ALS mice (B6.Cg-Tg(SOD1-G93A)1Gur/J) received intraspinal injections of either human bone marrow-derived MSC (bilateral injections of 1x105 cells per side in a volume of 1µl as described), or saline as vehicle control before symptom onset (day 40). Analysis of treatment effects consists in survival analyses, motor performance tests and immunohistochemical quantification of motor neuron loss and astrocytosis.

Results: MSC-conditioned medium (MSC-CM) as well as MSC co-culture has neuroprotective effects *in vitro*. MSC-culture decreases neuronal mRNA expression of glutamate receptor 2 and of matrix metalloproteinase 9 (MMP-9) in NSC-34 cells. Multiplex arrays reveal secretion of anti-inflammatory mediators and growth factors by MSC. First results in mutant SOD1 ALS mice show that intraspinal MSC injection increases survival and improves general condition and motor performance.

Discussion and conclusion: Mesenchymal stromal cells (MSC) are among the most promising candidates, based on their secretion of growth factors and cytokines and thereby generation of a neuroprotective microenvironment. The most efficient and tolerable route and frequency of administration require further clarification prior to translation into clinical evaluation.

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P270 RAT MESENCHYMAL STEM CELLS MODULATE FUNCTIONAL PROPERTIES OF PRIMARY CULTURED MICROGLIA VIA TGF-β SECRETION

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Keywords: microglia, mesenchymal stem cell, TGF-beta

Background: Previously, we reported that TGF-β levels in mesenchymal stem/stromal cells (MSCs) could be used as potential biological markers to predict the effectiveness of autologous MSC therapy in patients with amyotrophic lateral sclerosis. However, the underlying mechanism of TGF-β in MSCs was not fully elucidated in determining the functional properties of microglia. In this study, we aimed to clarify the role of TGF-β that is involved in MSC effectiveness, especially focusing on microglia functional properties that play a pivotal role in neuroinflammation.

Methods: Rat MSCs (5×10^5/mL, Invitrogen, CA, USA)) were plated into 6-well plates. After 24 hours, the medium was replaced and the cells were incubated for another 48 hours, at the end of which time (MSCs number; $1\text{-}2\times10^6$/mL) the medium was collected and centrifuged for obtaining MSC conditioned media (MSC-CM). The MSC-CM was treated for 24, 48, and 72 h to investigate effects of the releasing factors on microglia functional properties in LPS-stimulated microglia. The effect and mechanism were analysed with FACS, immunofluorescence study, qPCR, ELISA, and western blot.

Results: We found that MSC-conditioned media (MSC-CM) inhibited pro-inflammatory cytokine expression, restored alternative activated microglia phenotype (M2-like phenotype) markers such as fractalkine receptor (CX3CR1) and mannose receptor (CD206), and enhanced phagocytosis in LPS-stimulated microglia. In

addition, TGF-β in MSC-CM played a major role in these effects by inhibiting the NF-κB pathway and restoring the TGF-β pathway in LPS-stimulated microglia. Recombinant TGF-β (rTGF-β) also induced similar effects to MSC-CM in LPS-stimulated microglia.

Conclusion: We propose that MSCs can modulate the functional properties of microglia via TGF-β secretion, switching them from a classically activated phenotype to an inflammation-resolving phenotype. The latter role may be associated with the inhibition of neuroinflammatory processes in neurodegenerative disorders.

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P271 AUTOLOGOUS BONE MARROW MONONUCLEAR CELL INTRATHECAL TRANSPLANTATION MAY AFFECT THE SURVIVAL DURATION IN AMYOTROPHIC LATERAL SCLEROSIS – CLINICAL STUDY

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Keywords: stem cell therapy, survival, autologous bone marrow stem cells

Recent advances have shown cellular therapy as a promising treatment option for ALS. Animal studies have shown beneficial effects of cell therapy. This is a retrospective study of 75 patients with diagnosis of definite ALS who received intrathecal autologous bone marrow mononuclear cells transplantation. These patients also underwent standard rehabilitation and Riluzole. Kaplan-Meier Survival analysis was used to compute the survival duration. Analysis was done for various factors affecting survival such as type of onset, effect of age at onset, and lithium. Mean survival duration of patients in intervention group was 93 months which was higher than the mean survival duration of the control group. Survival duration of patients with onset of the disease below 50 years of age (107 months) was higher than in people with onset of the disease above 50 years of age (85 months). Survival duration of limb onset (101 months) was higher than bulbar onset (80 months). Survival duration of patients who were given lithium (104 months) after cell transplantation was significantly higher than the patients who were not given lithium (71 months). This difference between the lithium and non lithium group was statistically significant (p=0.041). Mean survival duration of the intervention group was also higher than the survival duration of ALS patients in previous epidemiological studies.

The limitation of this study is that it is not a prospective study. The control and the intervention group have not been randomised. The effect of cell therapy at various stages of the disease has not been studied. The effect of route of administration and dosage of injected cells on the outcome has not been analysed.

The cell therapy combined with rehabilitation, Riluzole and Lithium given at early stage may alter the course of the disease. Larger controlled trials with strict protocols are suggested to arrive at a definitive conclusion.

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P272 EXPANSION OF TREGS BY INTERLEUKIN-2/ANTIBODY COMPLEXES SLOWS DISEASE PROGRESSION IN MUTANT SOD1 MICE

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Keywords: T-cell, inflammation, interleukin

Background: The peripheral immune system is implicated in modulating glial cell activation, motor neuron survival and disease progression in ALS. Activation of T-regulatory cells (Tregs) may be a disease modifier of ALS and transplantation of Tregs is neuroprotective in mutant SOD1 mice. Specific stimulation of Treg expansion over harmful T-effectors was optimised using an interleukin-2 (IL-2)/IL-2 antibody (IL-2 mAb) complex with rapamycin (rapa). Here, we investigate the therapeutic potential of expanding endogenous Tregs using IL-2/IL-2 mAb complexes in mutant SOD1 mice.

Methods: SOD1G93A mice (n=10 male and n=10 female per group) were administered IL-2/IL-2 mAb complex (1 μg/5 μg) + rapa (1 mg/kg), rapa alone or vehicle 2-3 times weekly from 60 days of age. Mice were examined for weight loss, motor function and survival. Peripheral blood and spleens were analysed for T-cell populations using FACS. Spinal cords were analysed for motor neuron counts, astrocyte and microglial activation

using immunohistochemistry. Pro- and anti-inflammatory cytokine expression was determined by qPCR analysis of spinal cords.

Results: Treatment with IL-2/IL-2 mAb + rapa significantly prolonged survival of male SOD1G93A mice ($p<0.01$) without affecting disease onset, compared to rapa and vehicle groups. IL-2/IL-2 mAb significantly elevated CD4+ FoxP3+ Treg numbers by 6-fold in blood and 2-fold in spleens of SOD1G93A mice, but not CD8+ T-cells, confirming selective Treg expansion. Furthermore, these Tregs showed increased CTLA4 and GITR expression, consistent with a suppressor phenotype. Lastly, there was reduced glial cell activation in spinal cords of SOD1G93A mice treated with IL-2/IL-2 mAb + rapa.

Discussion and conclusions: We demonstrate IL-2/IL-2 mAb complexes efficiently stimulate selective expansion of suppressor Tregs in mutant SOD1 mice, leading to attenuated glial cell activation, inflammation and increased survival in mutant SOD1 mice. These data suggest that expansion of endogenous neuroprotective Tregs using this novel approach may be an effective and feasible therapeutic strategy for ALS.

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P273 METABOLIC AND IMMUNOLOGIC EFFECTS OF ANTI-INTERLEUKIN-6 RECEPTOR ANTIBODY (MR16-1) IN SOD1(G93A) MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: interleukin-6, Tregs, metabolism

Background: Interleukin-6 signaling is involved in the control of regulatory T cells (Tregs) differentiation and energy metabolism, which could represent relevant therapeutic targets in Amyotrophic Lateral Sclerosis (ALS). Indeed, blood Tregs are inversely associated with disease progression, and nutritional intervention showed promising results in ALS patients. MR16-1 is a monoclonal antibody directed against interleukin-6 receptor antibody, suppressing IL-6 effects in a dose dependent-manner.

Objective: To determine metabolic and immunologic effects of MR16-1 on a SOD1*G93A mutant mouse model.

Methods: 12 SOD1 mutant G93A mice (strain B6SJL-Tg(SOD1*G93A)1Gur/J) were treated intraperitoneally with MR16-1, 20 mg/kg twice a week, from 10th to 20th week post-natal. SOD1 mutant and wild-type mice received PBS with same regimen. For all mice, blood was collected at 4 time points and organs after sacrifice at 20th weeks post natal. Longitudinal behavioral analysis with rotarod test was performed, and body weight was regularly measured. CD4+CD25+FoxP3+ Tregs were counted in blood by flow cytometry analysis. Plasma, cortex, and muscle metabolomic analysis is scheduled and will use nuclear magnetic resonance spectroscopy and flow injection analysis coupled to a tandem mass spectrometry.

Results: Behavioral analysis has shown that treatment with MR16-1 did not slow disease progression. A slight increase in weight gain of SOD1 mutant mice was observed after 2 weeks of treatment ($p<0.05$) although from the 8th week of treatment to sacrifice, treated mutant mice showed a decrease in weight gain, in comparison with untreated mutants. After 2 weeks of treatment, blood Tregs count in treated mutant mice was decreased comparatively to untreated mutant mice ($p=0.03$). As for weight gain, this effect was reversed after 10 weeks treatment ($p=0.02$). Metabolomic data are still under process and will be available at the end of summer 2015.

Discussion: Initial decrease of Tregs count in SOD1 mutant treated mice is an unexpected result in regard to previous studies of MR16-1 in different mouse models.

Together with the reversal of this effect after 10 weeks treatment, it suggests that Tregs differentiation in ALS is linked to another phenomenon, such as microglia activation, on which MR16-1 treatment could interfere. We suggest that there are two different stages of clinical and immunological evolution, consistent with a modification in two steps of treated mutants body weight curves.

Conclusion: In this longitudinal study of SOD1 mutant mice treated with MR16-1, body weight slightly increased at 2nd week after beginning of treatment, and then decreased compared to untreated SOD1 mutant (from 8th to 10th week). Likewise, blood Tregs count decreased in a first step, and then increased in SOD1 mutant mice. While blocking the IL6 pathway does not seem to improve the clinical condition of mice, it provides us new hallmarks of the link between inflammation and metabolism in ALS.

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P274 ASTROCYTE-DERIVED TGF-B1 ACCELERATES DISEASE PROGRESSION IN ALS MICE BY INTERFERING WITH NEUROPROTECTIVE FUNCTIONS OF MICROGLIA AND T CELLS

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Keywords: TGF-β1, astrocytes, SOD1

Background: Transforming growth factor-β1 (TGF-β1) is a pleiotropic cytokine that has a key role in maturation and activation of microglia, proliferation and differentiation of T cells, and probably trophic support for motor neurons. Although TGF-β1 was increased in the plasma or CSF of ALS patients, its exact role in ALS pathogenesis remains unknown.

Objectives: This study is aimed to explore the roles of TGF-β1 in non-cell autonomous neurodegeneration mediated by glial cells.

Methods: TGF-β1 levels were examined in mutant SOD1 mice and sporadic ALS patients. $SOD1^{G93A}$ mice with astrocyte-specific overproduction of TGF-β1 ($SOD1^{G93A}$/TGF-β1) were generated using GFAP-TGF-β1 mice which overexpressed TGF-β1 in astrocytes, and disease phenotypes, alteration of glia/immune cells, and expression profiles of glia/immune-related molecules were analyzed. TGF-β1 levels were also examined in slowly progressive lox$SOD1^{G37R}$ mice with astrocytic deletion of mutant SOD1. Correlation between the survival times and expression levels of TGF-β1 in the lumbar spinal cords of $SOD1^{G93A}$ mice was examined. Efficacy of TGF-β signaling inhibitor SB-431542 in symptomatic $SOD1^{G93A}$ mice was evaluated. TGF-β signaling within the motor neurons was examined at different stages of $SOD1^{G93A}$ mice and end-stage $SOD1^{G93A}$/TGF-β1 mice.

Results: TGF-β1 levels were elevated in astrocytes of symptomatic mutant SOD1 mice and sporadic ALS patients, and astrocytic overproduction of TGF-β1 in $SOD1^{G93A}$ mice accelerated disease progression with reduced IGF-I production in deactivated microglia and fewer infiltrated T cells with a dysregulated IFN-γ/IL-4 balance. Astrocytic deletion of mutant SOD1 in lox$SOD1^{G37R}$ mice resulted in slowing disease progression with a decreased level of astrocytic TGF-β1. TGF-β1 levels negatively correlated with the survival times of $SOD1^{G93A}$ mice. Administration of TGF-β signaling inhibitor extended the survival of $SOD1^{G93A}$ mice. Defective TGF-β signaling within the motor neurons of $SOD1^{G93A}$ mice was observed before the disease onset and not restored by the overproduction of TGF-β1 in $SOD1^{G93A}$/TGF-β1 mice.

Conclusions: We identify astrocytic TGF-β1 as a determinant of disease progression of ALS through inhibiting the neuroprotective inflammatory response by microglia and T cells. Targeting glial TGF-β signaling may represent a novel disease-modifying therapy for ALS.

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P275 IBUDILAST, A PHOSPHODIESTERASE (PDE) 4 AND 10 AND MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) INHIBITOR, DEMONSTRATES EFFICACY IN TWO DROSOPHILA MELANOGASTER MODELS OF ALS

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Keywords: drosophila, SOD1, VAPB

Background: *In vivo* evidence implicating activated microglia in ALS pathophysiology and neuroinflammatory processes in disease progression suggests that ALS is a non-cell autonomous neurodegenerative disease (1). Ibudilast, a centrally active, small molecule compound, reduces activated microglia, inhibits neuroinflammation, and has strong neuroprotective properties in several disease models. Ibudilast inhibits phosphodiesterase (PDE) 4 and 10, macrophage migration inhibitory factor (MIF), and suppresses the production of pro-inflammatory cytokines and increases production of anti-inflammatory cytokines and various neurotrophic factors which may represent a novel pharmacotherapeutic approach in ALS treatment (2-4).

Objectives: This was a proof-of-concept study to evaluate ibudilast's effects on 2 specific functional phenotypes in the ALS fly model: paraquat sensitivity and viability. Two genes associated with ALS, superoxide dismutase (SOD1) and vesicle associated membrane protein (VAMP)-associated protein B (VAPB), show deficiencies such as hypersensitivity to oxygen radical generating compounds and larval lethality with rare adult escapers.

Methods: After it was established that wild-type flies had no food preference between DMSO (vehicle) and ibudilast, the experiment proper was begun. In the paraquat-induced toxicity assay, SOD1 mutant adult flies at 1 day of age were collected and kept on normal fly food for 24 h, then transferred to vials the next day with sucrose plus 2 mM paraquat in DMSO or 2) sucrose plus 2 mM paraquat plus the test compounds (0.2, 2, 20, or 30 mM ibudilast, 1 mM riluzole, or 25 IU/mL Vitamin E). Flies were quantified after 24 h. In the viability assay, VAPB

mutant flies at the 1st instar stage of larva development were administered 20 mM ibudilast, 1 mM riluzole, 25 IU/mL Vitamin E, or vehicle (DMSO) for 9 days and percent viability was assessed.

Results: Ibudilast dose-dependently ($P<0.005$) increased survivorship of the paraquat fed adult flies as effectively as riluzole and Vitamin E in the SOD1 model. Moreover, in the VAPB model assessing viability, the ability of larvae to develop into adult flies, ibudilast showed significant improvement ($P<0.001$). Neither riluzole nor Vitamin E showed any improvement.

Discussion and conclusions: In two *in vivo* models of ALS, SOD1 and VAPB, ibudilast demonstrated efficacy as a potential treatment for ALS. Ibudilast's ability to reduce oxidative stress in the SOD1 fly model and improve viability in the VAPB model lends further evidence to its neuroprotective and neuroimmodulatory actions.

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P276 INVESTIGATING UPPER MOTOR NEURONS IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: SOD1, brain, GDNF

Background: A lack of understanding exists regarding the role that upper motor neurons play in amyotrophic lateral sclerosis (ALS) disease pathology. Our work builds on the hypothesis that upper motor neurons are a crucial component of ALS that should be therapeutically targeted. We have previously shown that the motor cortex is important in initiating disease onset in the $SOD1^{G93A}$ (SOD1) rat model of ALS by knocking down mutant SOD1 in the motor cortex alone. The mechanisms underlying this phenomenon however, remain unknown. Here, we investigate upper motor neurons in the SOD1 rat by examining cortical electrophysiology and by directly targeting the motor cortex using human neural progenitors engineered to secrete glial cell line-derived neurotrophic factor (GDNF).

Methods: Transcranial magnetic stimulation of ALS patients has established that cortical hyperexcitability occurs early in disease and even at pre-symptomatic stages and is linked to neurodegeneration. To further explore the mechanisms underlying the brain's involvement in initiating disease pathology, we assessed the electrophysiological properties of cortical neurons in the SOD1 rat using acute cortical slice preparations.

As it has been shown that GDNF can protect motor neuron function and survival in models of ALS, we performed bilateral injections of human neural progenitor cells expressing GDNF (hNPC-GDNF) directly into the motor cortex of SOD1 rats to determine whether these cells had beneficial effects on motor function and survival.

Results: We found that cortical electrophysiological properties show signs of hypoexcitability in the SOD1 rat at a behaviorally pre-symptomatic time point of approximately 120 days.

hNPC-GDNF transplanted directly into the motor cortex of SOD1 rats survived and released GDNF, which was then taken up by surrounding neurons including corticospinal motor neurons. Critically, these targeted injections of hNPC-GDNF resulted in significantly delayed disease onset and extended survival.

Conclusions: The brain is a crucial component in the initiation of ALS disease onset. One possible mechanism underlying the brain's role in initiating disease onset in this ALS rat model is cortical hypoexcitability. Targeting the motor cortex of SOD1 rats with neural progenitor cells expressing GDNF leads to significant beneficial effects on motor function and extends survival.

Discussion: We are currently investigating the time course of altered electrophysiological properties to determine whether there is an early hyperexcitability followed by hypoexcitability that has been suggested in other models. The novel approach of therapeutically targeting cortical motor neurons has great potential for a successful ALS treatment. Furthermore, as the transplantation strategy proposed here does not involve altering mutant SOD1 expression, it could be relevant to sporadic ALS cases and not just the genetically predisposed population harbouring SOD1 mutations. Targeting the brain is much less invasive than targeting other affected central nervous system regions such as the spinal cord, making it ideal for fast translation to the clinic.

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P277 THE DISULPHIDE INTERCHANGE ACTIVITY AND GENERAL CHAPERONE FUNCTION OF PROTEIN DISULPHIDE ISOMERASE (PDI) ARE BOTH PROTECTIVE AGAINST CELL DEATH PATHWAYS IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Keywords: Protein Disulphide Isomerase, endoplasmic reticulum stress, protein inclusions

Background: Increasing evidence is arising regarding endoplasmic reticulum (ER) stress as a key contributor to Amyotrophic Lateral Sclerosis (ALS) pathogenesis. Protein Disulphide Isomerase (PDI) is a protective ER chaperone which functions by refolding misfolded proteins, and thus decreases inclusions, a major feature of ALS. It is unclear, however, whether PDI carries out this protection via its disulphide interchange activity or general chaperonic function. We therefore investigated a number of PDI variants; wildtype PDI, PDI without its disulphide interchange activity (PDI QUAD), and PDI localised to the cytoplasm, for their effectiveness to down-regulate the activation of cell death proteins, Bax and Caspase-3.

Objectives: The aim of this study was to determine what function of PDI pertains to its protective effect and whether PDI's localisation to the cytoplasm affects this protection.

Methods: A Neuro-2A cell line was used to mimic ALS disease. Cells were co-transfected with mutant $SOD1^{A4V}$ and one of the PDI variants (PDI wildtype, PDI QUAD, cytoplasmic PDI wildtype, cytoplasmic PDI QUAD) for 72 hours before analysis of its effect on Bax activation, Bax recruitment to the mitochondria and Caspase-3 activation using immunofluorescence microscopy.

Results: The proportion of Neuro-2A cells with Bax activation was significantly ($P<0.001$) reduced when subjected to either PDI wildtype or PDI QUAD compared to cells solely transfected with $SOD1^{A4V}$. Both PDI and PDI QUAD also significantly decreased Bax recruitment to the mitochondria ($P<0.01$) and Caspase-3 activation ($P<0.01$ and $P<0.001$ respectively) compared to $SOD1^{A4V}$ transfected cells. In addition, the cytoplasmic PDIs were also able to significantly down-regulate the activation of Bax and Caspase-3 and Bax recruitment.

Discussion and conclusions: PDI without its disulphide interchange activity was protective against cell death protein activation. Previous studies, however, have demonstrated that PDI without its disulphide interchange activity does not decrease ER stress in ALS cell models. This suggests that PDI's chaperone function may be protective against alternative pathways leading to motor neuron death in ALS. Both cytoplasmic PDI proteins also showed a protective effect, suggesting that PDI does not have to be limited to the ER to refold misfolded proteins. These findings may be useful when designing therapeutic drugs that mimic PDI in order to treat ALS.

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P278 INHIBITION OF P38 MITOGEN ACTIVATED PROTEIN KINASE INCREASE MOTOR NEURON SURVIVAL AND DELAY DISEASE ONSET IN ALS TRANSGENIC MICE

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Keywords: p38 MAPK inhibitor, extrinsic apoptosis pathway, Fas

Recently, motor neuron specific Fas-dependent cell death pathway, Daxx-p38a-NO feedback amplication loop, is known to be crucial for a motor neuron cell death in ALS. In this study, we searched for the potential neuroprotective effect of p38 MAPK inhibitor, semapimod, in *in vivo* ALS model. G93A-SOD1 transgenic mice were divided in 2 groups; control and p38 MAPK inhibitor (semapimod) treated groups. Clinical status, rotarod test, and survival rates of each group of mice were evaluated. Further biochemical study was performed for motor neuron counting and cell survival signaling. Semapimod, a p38 MAPK inhibitor suitable for clinical use, significantly delayed disease onset by 7 days and rescued the motor neurons in ventral horn, however survival did not differentiate from the controls. It may be that semapimod inhibits motor neuron death efficiently, but is not able to restore the function of the entire motor unit in order to affect survival. There might be some threshold that motor neuron decrement affects survival. Loss of motor neuron function above that threshold might results semapimod treated groups to catch up the control and follow the inevitable course of ALS.

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P279 MAHOGUNIN RING FINGER 1 CONFERS CYTOPROTECTION AGAINST MUTANT SOD1 AND IS DEFECTIVE IN ALS MICE

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Keywords: SOD1, autophagy, ubiquitin-ligase

Background: Accumulation of misfolded, disease-linked proteins is involved in the pathomechanism of motor neuron disease. The precise protein quality control system by Mahogunin ring finger-1 (MGRN1), E3 ubiquitin protein ligase that catalyzes mono-ubiquitination against misfolded proteins including mutant SOD1 in neurons remains elusive.

Objectives: The aim of this study is to reveal the neuroprotective potential and mechanisms of MGRN1.

Methods: Using the cultured cells and tissue from SOD1 mice, the levels of MGRN1 and interaction of MGRN1 with SOD1 and other key proteins in protein quality control were examined. Moreover, neuroprotective potential of MGRN1 against mutant SOD1-mediated toxicity was evaluated.

Results and discussion: MGRN1 was dysregulated in the cellular and mouse models overexpressing mutant SOD1 and preferentially interacted with mutant SOD1. Moreover, MGRN1 promotes the degradation of mutant SOD1 and protects cells against mutant-mediated toxicities partly through autophagy pathway in the cells. MGRN1 also alleviates mutant-mediated toxicities from the cultured cells.

Conclusion: Our findings indicate that MGRN1 selectively targets misfolded SOD1 proteins for degradation and MGRN1-mediated protein quality control mechanism is a novel candidate for therapeutic target in neurodegenerative diseases including ALS.

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P280 MUTATED SOD1 SILENCING IN ASTROCYTES: MECHANISMS LEADING TO THE PROTECTION OF THE NEUROMUSCULAR FUNCTION IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: gene therapy, astrocytes, Superoxide dismutase 1 (SOD1)

Years of research on animal and cellular models of ALS have revealed a complex array of cellular dysfunctions leading to muscle denervation and motor neuron (MN) death. Extensive experiments, predominantly conducted on mouse models overexpressing a mutated form of the superoxide dismutase 1 (SOD1) protein, highlight the important contribution of glial cells. Astrocytes have been shown to be mainly involved in ALS progression. However, the molecular mechanisms underlying the pathogenic interaction between astrocytes and MNs remain elusive.

Using adeno-associated viral (AAV) vectors combined with cell-type specific promoters, we were able to selectively target either MNs or astrocytes, and express a microRNA to suppress human SOD1 expression by RNA interference. Using this approach in a mouse model overexpressing $SOD1^{G93A}$, we obtained prominent therapeutic effects when the treatment was administered either in neonatal mice or at an early stage of the disease (1). Our current work aims at elucidating the non-cell-autonomous effects of suppressing human SOD1 in astrocytes, in the context of this gene therapy approach against ALS.

Human SOD1 was downregulated in astrocytes along the entire spinal cord by performing intracerebroventricular injections of the AAV9-gfaABC$_1$D:gfp-miRSOD1 vector at postnatal day 3 (PND3). Following injection, neuromuscular function was monitored by measuring Compound Muscle Action Potentials (CMAP) in the *triceps surae*. The occupancy of the neuromuscular junctions (NMJ) and MN survival were analyzed *postmortem* at early (PND65) and late stages (PND100) of the disease.

We found that mutated SOD1 silencing in astrocytes does not prevent the decline of CMAP values during the early phase of the disease. Nevertheless, we later observed a clear effect on CMAP values, which progressively increased in treated ALS mice to reach amplitudes that were similar to wild-type mice. Consistent with this effect, we noticed that NMJ innervation was significantly preserved at PND100 in the treated ALS mice, recovering from the denervation observed at PND65. These behavioral and histological data suggest a neuroprotective effect of mutated SOD1 silencing in astrocytes. The treatment leads to a significant recovery of the neuromuscular function, most likely by promoting muscle reinnervation from the MNs surviving until late disease stages.

In order to investigate the molecular and cellular mechanisms leading to this late neuroprotection, we are currently performing a gene expression analysis of the lumbar MNs at early and late stages of the disease, in the condition where SOD1 is downregulated in astrocytes. Our objective is to explore the pathologic interaction between astrocytes and MNs in order to possibly unravel new therapeutic targets for ALS.

Acknowledgments: The authors thank Vivianne Padrun, Fabienne Pidoux, Christel Sadeghi, Philippe Colin, and Aline Aebi for technical assistance.

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P281 IMMUNOTHERAPEUTIC TARGETING OF MONOMER/ MISFOLDED SOD1 IN A CANINE DISEASE MODEL OF ALS

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Keywords: disease models, misfolded SOD1, therapeutic strategy

Background: Mutations in *SOD1* cause misfolding of the SOD1 protein, exposing a pathological epitope, *SODI-Exposed-Dimer-Interface* (SEDI). An active immunization strategy in an ALS mouse model with a SEDI peptide reduced levels of misfolded SOD1 in the spinal cord, leading to a delay in disease onset and extending survival (1). Canine degenerative myelopathy (DM) is a naturally-occurring, adult-onset, neurodegenerative disease that has many similarities to human ALS. Homozygosity for the *SOD1* missense mutation, *SOD1:c.118G>A,* underlies most cases of canine DM. The homogeneity in clinical progression of canine DM will facilitate translation of therapies into human applications. We have verified that the SEDI epitope is present in DM-affected dogs and propose that active immunization with peptide corresponding to the SEDI epitope will significantly delay disease progression and extend survival in dogs with DM.

Objectives: 1) Evaluate the safety of immunotherapy targeting misfolded SOD1 as a *short-* and *long-term* study; 2) Evaluate preliminary therapeutic effects of immunotherapy targeting misfolded SOD1 on canine DM.

Methods: 1) We utilized 8 normal dogs in a research colony at the University of Missouri segregating the *SOD1* mutation, for which 2 dogs were homozygous. Using a cross-over design, dogs were immunized intramuscularly with SEDI peptide or adjuvant on days 0, 14, 35, 56, 84 and 119 and monitored through day 299. 2) A cohort of 5 DM affected dogs showing early signs of weakness/ataxia were entered into a single-arm (no control arm), non-randomized phase II trial. Functional measures of disease were used to evaluate neurologic status, and upper and lower motor neurons.

Results: 1) Six of 8 dogs developed antibody titers to misfolded SOD1 (SEDI peptide). No significant differences were detected on CBC, plasma biochemistry, urinalysis and CSF analysis. Longitudinal assessment of a variety of immune parameters in peripheral blood showed no significant differences between adjuvant and SEDI immunized groups with respect to mean % B cells, CD4+ and CD8+ T cells, and T regulatory cells; CD4+ lymphocytes expressing IFN-g or IL-17; or mean total lymphocyte counts. Dogs receiving SEDI peptide had significant (P=0.05) decreases in mitogen-induced T cell proliferation. 2) The median time to onset of nonambulatory paraparesis was longer (13 m) in immunized dogs compared to historical controls (10 m) although this difference was not significant (Log-rank P=0.93). MRI and electrodiagnostic studies are ongoing.

Discussions and conclusions: SEDI peptide immunization has no adverse effects with variable antibody responses. We were unable to detect differences between SEDI immunized and control in peripheral B cells and T cell subsets. This study in DM-affected dogs showed continued disease progression but may not be statistically powered to detect a difference.

Acknowledgements: ALS Association (Grant #2431).

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P282 TREHALOSE DECREASES MUTANT SOD1 EXPRESSION AND ALLEVIATES MOTOR DEFICIENCY IN EARLY BUT NOT END-STAGE AMYOTROPHIC LATERAL SCLEROSIS IN A SOD1-G93A MOUSE MODEL

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Keywords: trehalose, motor deficiency, autophagy

Background: Studies indicate that enhancing autophagy in mouse models of neurodegenerative disease can ameliorate the behavioral symptoms and pathological damage associated with the accumulation of pathological mutant proteins such as mutant superoxide dismutase (SOD1) (1-3).

Objectives and methods: This study investigated the effects of trehalose treatment on both early and end-stage disease in a transgenic mouse model of ALS via short-term (30 days after administration) and long-term (from 60 days after administration to death) trehalose treatment experiments. Sixty-day-old female SOD1-G93A transgenic mice were treated daily with 2% (w/v) trehalose in their drinking water for 30 days and monitored until they reached a neurological score of four, whereupon they were euthanized by cervical dislocation. Neurological, rotarod performance test and hanging wire test scores were recorded and body weight monitored. After death, the spinal cord was removed to assess the number of motor neurons and to measure the expression of mutant SOD1, LC3-II and p62.

Results: Trehalose significantly reduced the levels of mutant SOD1 and p62 and increased LC3-II in the spinal cords of 90-day-old SOD1-G93A transgenic mice. Furthermore, trehalose treatment significantly postponed disease onset, lengthened the time it took to reach a neurological score of 2 and preserved motor function; however, trehalose became less effective at delaying further disease progression as the disease progressed beyond a neurological score of 2 and it failed to extend the survival of SOD1-G93A transgenic mice. Additionally, independent of autophagy, trehalose consistently inhibited microgliosis and astrogliosis throughout the entire duration of the study.

Discussion and conclusions: Trehalose may be a useful add-on therapy in conjunction with other ALS treatment options to alleviate symptoms in early-stage.

Acknowledgements: This work was supported by the Natural Science Foundation of China (grant numbers 31371089 and 81171210).

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P283 NEUREGULIN1 AS A THERAPEUTIC TARGET TO MODULATE MICROGLIAL ACTIVATION IN ALS

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Keywords: neuregulin1 antagonist, therapeutic target, disease progression

Background: Neuregulin1 (NRG1) is a gliotrophic factor that regulates glial development and survival, synaptogenesis, axoglial interactions, and microglial activation. We recently found that NRG1 receptors are activated on microglia in the ventral horn of both ALS patients and in ALS-SOD1 mice (1). NRG1 signaling is also activated on microglia in the corticospinal tracts in ALS patients with predominant upper motor neuron signs (2), suggesting a common pathological mechanism (1,2). We have developed a targeted NRG1 antagonist called HBD-S-H4 that when given intrathecally, reduced microglia activation in a rat chronic spinal cord pain model. Therefore, here we hypothesize that neuregulin1, as a therapeutic target, modulates microglia in ALS.

Objectives: To determine how NRG1 in the central nervous system (CNS) modulates microglial activation to slow disease progression in the ALS-SOD1 mouse model.

Methods: To deliver HBD-S-H4 to the CNS, we generated triple transgenic (Tg) mice (GFAP-tTA:tetO-HBD-S-H4:SOD1*G93A) to express HBD-S-H4 in the CNS. In an alternative therapeutic approach, we injected HBD-S-H4 through an implanted intracerebroventricular (icv) cannula at different stages of the disease in SOD1 mice. Dose response and time course of HBD-S-H4 for microglial activation were tested in CX3CR1 Tg mice. Body weight, disease onset and progression, animal survival as well as pathological changes were measured in the triple Tg mice, HBD-S-H4 treated SOD1 mice and compared with their respective control groups.

Results: Our data shows that the expression of HBD-S-H4 in the CNS improves early chronic motor performance deficits, delays disease onset and prolongs survival in GFAP-tTA:tetO-HBD-S-H4:SOD1 mice compared with GFAP-tTA:SOD1 as well as SOD1 mice. Consistent with this therapeutic effect of transgenic expression of HBD-S-H4, we found that higher levels of HBD-S-H4 expression correlate with longer survival. The effect of HBD-S-H4 icv treatment showed dose dependent response on microglial activation in CX3CR1 Tg mice. Weekly icv treatment of recombinant HBD-S-H4 up to 14 weeks in the CNS had no toxic effects and was initially found to delay disease onset and prolong survival in the SOD1 mice. Measurements of the cellular pathology in GFAP-tTA:tetO-HBD-S-H4:SOD1 Tg mice and HBD-S-H4 icv-treated SOD1 mice are currently underway.

Discussion and conclusions: We have identified a common therapeutic target of NRG1 receptor activation on activated microglia in both ALS patients and the ALS-SOD1 mouse model. We are currently testing how HBD-S-H4 functions by modulating microglial activation in the SOD1 mouse model and whether this would be a potential therapeutic treatment for patients with ALS.

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P284 ANTISENSE OLIGONUCLEOTIDES FOR SOD1 IMPROVES FUNCTION AND EXTENDS LIFE OF SOD1-G93A MICE

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Keywords: SOD1, antisense oligonucleotide, mouse

Background: Dominantly inherited ALS can be caused by mutations in the Cu/Zn superoxide dismutase (SOD1) gene. Compelling evidence suggests that SOD1-toxicity requires protein expression. Therefore, reduction of SOD1 protein within the CNS ought to be beneficial. Centrally administered antisense oligonucleotides (ASOs) provide an effective method for reduction of SOD1. Here we report on the development of potent ASOs for SOD1 and their impact on SOD1 in rodent models of ALS.

Objective: To identify potent SOD1 ASOs which could be safely delivered to the CNS of rodents and evaluate their efficacy.

Methods: ASOs were identified by treating A431 cells with ASOs and quantifying SOD1 mRNA knockdown. Candidate ASOs were tested in SOD1-G93A mouse and rat models. For mice, ASOs were injected intracerebroventricularly (ICV), for rat, by intrathecal injection. SOD1 mRNA was measured by Taqman assay. Aif1 and GFAP mRNA were likewise measured as estimates of microglial and astrocytic reactivity. Suppression of SOD1 protein was quantified by ELISA. For efficacy assessment, female C57Bl6 congenic SOD1-G93A high copy mice were injected with vehicle or one of three ASOs targeting SOD1. For survival and behavioral studies, mice were injected at day 50 and day 95 with 300 μg of ASO (n=20 per treatment group). Body weight and rotarod performance were monitored as were clinical scores of motor function. Compound motor action potentials (CMAPs) were measured in a second cohort of mice (n=12 per group) injected once at day 35. All injections and data collection were completed in a blinded fashion.

Results: *In vitro* screening identified potent ASOs, which suppressed SOD1 mRNA with an IC50 <1 μM in A431 cells. Dose ranging studies in SOD1-G93A rat showed knockdown of SOD1 mRNA in the lumbar cord, with an IC50 of 1 μg/g tissue. SOD1 mRNA was reduced by more than 50% up to 8 weeks following a single injection. No behavioral changes were noted following injection and Aif1 and GFAP were not elevated. In the efficacy studies, the ASOs delayed body weight and clinical scoring changes to different extents. The median onset in the vehicle treated group was 126 days, with ASO treated groups showing improvement by 16 to 28 days. Median survival in ASO treated animals ranged from 180 to 246 days ($p < 0.001$; vehicle survival of 174). Lastly, a single injection of vehicle preserved CMAP function out to week 17.

Discussion: These data suggest that centrally-delivered ASOs can safely suppress SOD1 toxicity in SOD1-G93A mice and rats, delaying disease onset and progression. The benefits appear durable, consistent with the half-life of the ASOs. These findings support assessing these and similar ASOs for their safety profile for possible use in clinical studies in SOD1-linked ALS patients.

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P285 GENETIC CORRECTION OF C9ORF72 REPEAT EXPANSION MUTATION IN ALS/FTD PATIENT IPSCS

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Keywords: C9ORF72 repeat expansion, iPSCs, CRISPR

Background: A hexanucleotide (G4C2) repeat expansion in chromosome 9 open reading frame 72 (C9ORF72) gene is the commonest single identified genetic cause of both familial and sporadic amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD).

Objectives: We have used ALS patient derived induced pluripotent stem cells (iPSC) carrying the repeat expansion mutation and applied the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 gene editing technology to correct the abnormal repeat expansion and to replace it with the wild type number of repeats, to create an isogenic control for the patient line.

Methods: Induced pluripotent stem cell (iPSC) lines were generated from four ALS patients carrying the repeat expansion mutation in the C9ORF72 gene. Three iPSC lines were generated from each patient and were demonstrated to be karyotypically normal. One ALS/FTD patient iPSC line was used for gene editing to target the expanded G4C2 repeats using CRISPR/Cas9-mediated homologous recombination (HR), in the presence of a plasmid DNA donor template containing a puromycin cassette for positive selection of the corrected clones. Puromycin resistant clones were assessed by repeat-primed PCR (RP-PCR), and then evaluated for targeted integration by direct sequencing.

Results: Twenty four out of one hundred clones showed no repeat expansion by RP-PCR and accurate insertion of the wild type repeats by direct sequencing.

Discussion and conclusion: To the best of our knowledge, this is the first study generating an isogenic control for any repeat expansion disease using CRISPR technology. These results demonstrate that the CRISPR/Cas9 system can be used for gene editing of repeat expansion mutations. Motor and cortical neurons from these iPSC lines will be a valuable tool with which to understand the pathogenesis of the repeat expansion and to develop possible therapies.

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P286 4-AMINOPYRIDINE INDUCES ACTIVITY AND RESCUES HYPOEXCITABLE MOTOR NEURONS FROM MUTANT FUS AND SOD1 ALS PATIENT-DERIVED IPSC

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Keywords: iPSC, hypoexcitability, FUS

Despite intense research on widely established amyotrophic lateral sclerosis (ALS) animal models the translation of treatment options remains ineffective. In the present study, we investigated pathophysiological mechanisms underlying ALS in functional motor neurons (MNs) differentiated from induced pluripotent stem cells (iPSC) of healthy controls and ALS patients carrying mutations in the FUS or SOD1 gene. Patient-derived MNs were less active and excitable compared to healthy controls, at least partly due to reduced Na^+/K^+ ratios in both ALS groups and elevated potassium receptor expression levels in the FUS group only. FUS and SOD1 iPSC-derived MNs both showed elevated endoplasmic reticulum stress (ER) levels and a higher number of MNs that underwent apoptosis. Treatment with the FDA approved voltage-gated potassium channel antagonist 4-Aminopyridine (4AP) restored ion-channel imbalances, increased neuronal activity levels and decreased ER stress and MN degeneration. This study provides novel pathophysiological data including a mechanistic explanation for the observed hypoexcitability in patient-derived MNs and a new therapeutic strategy to treat functional deficits and to provide neuroprotection in ALS.

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P287 SIGMA 1 RECEPTOR ACTIVATION MODULATES ER-MITOCHONDRIA CROSSTALK IN THE SOD1 MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: ERMCC, ER stress, motor neuron culture

Background: Motor neurons are vulnerable to disturbance in ER-mitochondria calcium cycle (ERMCC), the key player in the pathophysiology of ALS (1,2,3). The ER supplies Ca^{2+} to mitochondria through IP_3R at close contacts between the two organelles, the mitochondria-associated ER membrane (MAM). The Sigma 1 receptor (Sigma1-R) resides at MAM where it acts as an inter-organelle signaling modulator by stabilizing IP_3R. Mutations in *SIGMAR1* have been identified in ALS-FTD. Accumulation of Sigma-1R was found in motor neurons of ALS patients not linked to *SIGMA1R* mutations (4). Pharmacological manipulation of the receptor was neuroprotective in G93A hSOD1 and wobbler mice. Therefore, Sigma1-R modulation may provide a treatment option in ALS.

Objectives: The aims of this study in missed motor neuron co-cultures were (i) to elucidate distribution and activity of Sigma1-R in the presence and absence of mutated hSOD1, and (ii) to determine the pharmacological effects of Sigma1-R modulation on survival in native and hSOD1motor neurons and non motor neurons.

Methods: Embryonic mouse spinal motor and non-motor neurons were seeded on a glial feeder layer. Neuronal survival was assayed 12h after treatment of cultures with kainate and Sigma1-R agonists PRE-084 and SA 4503. Cytosolic Ca^{2+} dynamics were analyzed by single cell live calcium imaging using fura 2-AM. The cells were repetitively stimulated with kainate and bradykinin in the presence and absence of Sigma1-R agonists. Expression and mRNA values of Sigma1-R are examined by immunocytochemistry and qRT-PCR.

Results: PRE-084 significantly increased kainate induced Ca^{2+} response in motor neurons in hSOD1 neurons. Bradykinin induced Ca^{2+} response was significantly reduced in hSOD1 neurons. SA 4503 rescued altered bradykinin induced Ca^{2+} response in hSOD1 neurons. Both agonists did not influence viability of neurons in non-transgenic and SOD1 cell cultures and they showed no rescue effect against kainate induced toxicity.

Conclusion: Activation of Sigma1-R influences ERMCC, but it addresses different roles of the receptor in non-transgenic and G93AhSOD1 neurons. The Sigma1-R expression data will help us to understand the dual role of the receptor, and elucidate whether the expression pattern is affected by mutated SOD1 or/and pharmacological activation of the receptor. Stabilizing

ER-mitochondria interplay as key pathway will provide new treatment options in genetically different ALS forms.

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P288 CELL CYCLE INHIBITOR AMELIORATES MOTOR NEURON DEGENERATION INDUCED BY POLYGLUTAMINE-EXPANDED ANDROGEN RECEPTOR

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Keywords: spinal and bulbar muscular atrophy, cell cycle, TGF-beta

Background: Spinal and bulbar muscular atrophy (SBMA) is an adult-onset motor neuron disease caused by the expansion of a CAG trinucleotide repeat, which encodes a polyglutamine tract, in androgen receptor (AR) gene. Our previous study showed that the polyglutamine-expanded AR protein, the causative protein of SBMA, inhibits TGF-beta signaling, and thus induces the expression of cell cycle regulators, such as cyclines, E2F1, and PCNA, and hyper-phosphorylation of pRb protein, in the motor neurons of a transgenic mouse model of SBMA (AR-97Q).

Objectives: The aim of this study is to test the effects of flavopiridol, a cyclin-dependent kinase inhibitor that inhibits cell cycle, in the mouse model of SBMA.

Methods: Flavopiridol was intraventricularly administered to male AR-97Q transgenic mice via an osmotic pump. SD-208, an inhibitor of TGF-beta was intraventricularly administered to male wild-type mice via an osmotic pump. Rotarod performance was assessed weekly using an Economex Rotarod, while the grip strength was measured with a Grip Strength Meter. The loss of motor neurons and gliosis were evaluated using immunohistochemistry. Cell cycle re-entry in mouse neurons was assessed using BrdU.

Results: Intraventricular administration of flavopridol suppressed the expression of cell cycle markers, inhibited BrdU uptake in motor neurons within the spinal anterior horn and brainstem. Rotarod performance, grip power, and life span of AR-97Q mice (n = 20 for each group) were also substantially improved by flavopiridol, whereas this agent showed no detectable effects on the motor performance of wild-type mice. In contrast, pharmacological inhibition of TGF-beta induced the expression of cell cycle markers in spinal and brainstem motor neurons in wild-type mice (n = 6 for each group).

Discussion and conclusions: The present study showed that cell cycle re-entry is associated with the pathogenesis of neurodegeneration in SBMA, and cyclin-dependent kinase inhibitor inhibits motor neuron degeneration by the polyglutamine-expanded AR protein.

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Katsuno M, Adachi H, Minamiyama M, et al. J Neurosci. 2010; 30(16): 5702-5712.

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P289 GENOTYPE SPECIFIC IMPACT OF NUCLEAR FACTOR E2-RELATED FACTOR 2 (NRF2) TREATMENT IN ANIMAL MODELS OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: H63D HFE, SOD1(G93A), Nrf2

Background: H63D HFE gene variant, found in approximately 30% of ALS patients, impacts disease processes implicated in amyotrophic lateral sclerosis (ALS). In the double transgenic mouse model (SOD1/H67D), which is genetically relevant to ALS patients carrying H63D HFE, the addition of H67D HFE to SOD1 mice shortens survival and accelerates disease progression in association with elevated oxidative stress and decreased Nrf2 levels. The Nrf2 signaling pathway is the major cellular defense mechanism against oxidative stress and regulates expressions of many endogenous antioxidant genes. Neuroprotective effects of the Nrf2 activator 2-Cyano-3,12-Dioxooleana-1,9-Dien-28-Oic acid trifluoroethylamide(CDDO-TFEA) are reported in a number of preclinical models of neurodegenerative disease.

Objectives: To determine the impact of HFE genotype on the effect of CDDO-TFEA using double transgenic and SOD1 mice. We hypothesize a genotype-dependent response.

Methods: At 97-days of age, double transgenic and SOD1 mice were fed either a control diet or one supplemented with CDDO-TFEA (400mg/kg body weight). Effects on disease onset, disease progression and survival were evaluated. Disease onset was determined by motor performance on a rotarod. A gripstrength meter measuring forelimb and hindlimb strength was used to evaluate disease progression. Disease end-stage was used as a marker for survival, and was defined as the inability of an animal to right itself within 30 seconds after being placed on its side. Kaplan-Meier analysis, one-way ANOVA and repeated measures ANOVA were used to analyze survival, disease onset and disease progression. $p < 0.05$ was considered significant.

Results: Diet supplemented with CDDO-TFEA significantly extended median survival of double transgenic mice compared to control diet (149 vs. 143 days; $p = 0.027$; n = 13-18 per group) but CDDO-TFEA had no effect in SOD1 mice (survival 140 days vs. control - 138 days; n = 14 per group). Median survival of double transgenic mice (149 days) fed CDDO-TFEA diet was significantly longer than the SOD1 mice fed CDDO-TFEA diet (140 days; $p = 0.005$), but CDDO-TFEA had no effect on disease onset. Average age at onset for double transgenic mice on CDDO-TFEA and control diet was 116.2 ± 2.02 and 113.2 ± 1.56 days, whereas for SOD1 mice on CDDO-TFEA and control diet was 113.7 ± 1.41 and 114.2 ± 2.27 days.

Discussion and conclusions: A genotype-dependent response to Nrf2 activator treatment was identified, with CDDO-TFEA treatment significantly prolonging survival in double transgenic mice, but having no effect in SOD1 mice. This supports stratification of outcomes from clinical trials according to HFE genotype, and has potential implications for treatment strategies in clinical practice.

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P290 SYSTEMIC ANGIOGENIN DELIVERY REVERSES DEFECTS IN CAPILLARY DENSITY IN SOD1G93A MICE AND EXTENDS LIFESPAN IN FUS (1-359) MICE

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Keywords: Angiogenin, vasculature, FUS(1-359)mice

Background: Loss-of-function mutations in the Angiogenin (ANG) gene have been identified in patients with familial and apparently 'sporadic' forms of ALS[1]. Moreover, Angiogenin is enriched in motoneurons and transcriptionally activated and secreted under ischemic/metabolic stress. Motoneuron-derived Angiogenin is endocytosed by astroglia[2] (and potentially endothelial cells). Previous work from our group has identified ANG as a protective factor for motoneurons[3], and daily i.p. injections of ANG protein increase lifespan and delay onset of symptoms and motor function deficiencies in $SOD1^{G93A}$ mice[4].

Objective: To further develop ANG as a therapeutic we aim to assess the effect of ANG treatment on vascular integrity *in vivo*, and determine whether an increase in angiogenesis relates to the protective activity of ANG. Furthermore, we will determine the therapeutic effect of ANG treatment in a new mouse model of ALS, FUS (1-359) mice.

Methods: To assess changes in vascularization, ANG (1 μg dose, 3 times/week) was administered from symptom onset (90 days) until 115 days, a treatment that increased survival and delayed motor dysfunction in sex- and litter-matched $SOD1^{G93A}$ mice assessed according to the Ludolph guidelines. Serum ANG levels were determined by ELISA. Lumbar spinal cords were collected, sectioned (16 μm serial sections; L1-L5) and vasculature structure assessed via podocalyxin staining (marking endothelial cells). Stereological measurements of vessel number, density and volume were determined. Further ANG (1 μg dose, 3 times/week) was administered to FUS (1-359) mice from 70 days onwards. Weight measurements and motor function performance (via Rotarod and stride length analysis) were examined throughout disease progression.

Results: Increased ANG serum levels were detectable 2, 4 and 24 h after i.p. injection. ANG (1 μg dose, 3 times/week) treatment resulted in improved maintenance of vascular integrity/neovascularization in $SOD1^{G93A}$ mice in comparison to vehicle treated groups. Additionally, ANG treatment (1 μg dose, 3 times/week) from 70 days onwards improved survival outcome in FUS (1-359).

Discussion and conclusions: Our data strengthen ANG as a possible therapeutic for ALS.

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P291 A HIGH CALORIC DIET LEADS TO EXTENDED LIFESPAN, MOTOR DYSFUNCTION AND LUMBAR SPINAL CORD MOTOR NEURON LOSS IN TDP-43A315T MICE

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Keywords: mouse-models, TDP-43, diet

Background: Transgenic transactivation response DNA-binding protein 43 (TDP-43) mice expressing the A315T mutation under the control of the murine prion promoter progressively develop motor function deficits[1]; however, pre-mature sudden death due to intestinal obstruction halts disease phenotype progression in 100% C57Bl6/J congenic TDP-43 mice[2]. Recently, a jellified diet was reported to abolish this sudden death in TDP-43^{A315T} mice[3].

Objective: to compare standard pellet chow to a high moisture jellified diet and a high caloric jellified diet in TDP-43^{A315T} mice.

Methods and results: TDP-43^{A315T} mice (male only) were sustained on a standard pellet diet *(58% carbohydrate, 24% protein, 18% fat and 0% moisture*, n=16), a surgical recovery jellified diet (76a) *(18% carbohydrate, 4.7% protein, 1.5% fat and 74% moisture*, n=10) or a hypercaloric jellified diet (boost) *(37.8% carbohydrate, 9.9% protein, 21.6% fat and 30% moisture*, n=16) and life span compared (Kaplan-Meier survival analysis). The hypercaloric diet (boost) prevented sudden death and significantly extended survival (147 $\pm$ 27 days) in the TDP-43 mice compared to the standard pellet diet (102 $\pm$ 19 days) or jellified diet 76a (71 $\pm$ 7 days). These survival rates indicate that a high caloric diet (with a higher fat percentage than protein) is more important than a high moisture content diet. Disease progression was investigated via weight, Rotarod and stride length analysis and motor neuron survival analysed by Nissl stain counts. We deciphered post-natal day (PND) 80 as the disease onset time point. Decreased motor function performance and depleted motor neuron number indicated PND 120 as a late stage disease progression time point. PND 150 was the average end stage time point.

Discussion and conclusions: Our work suggests that a hypercaloric boost diet rather than a high water content jellified diet vastly improves the utility of the TDP-43^{A315T} model and that motor dysfunction observed is associated with lumbar loss of motoneurons. With this knowledge, future pre-clinical studies of therapeutics in this mouse model can be greatly improved.

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P292 MCT1 METABOLIC SUPPORT IN ALS DISEASE PATHOGENESIS

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Keywords: oligodendrocyte, metabolic support, MCT1

Background: Besides myelination, we and others recently discovered that oligodendroglia provide essential metabolic support to neurons through the expression of a particular monocarboxylate transporter (MCT) MCT1. Loss of MCT1 leads to slow neuronal degeneration. In the spinal cord of human ALS patients and SOD1G93A mice, we previously found that gray matter oligodendrocyte function is significantly affected in terms of myelination as well as MCT1 metabolic support. In SOD1G93A mice, oligodendrocytes degenerate and die and are replaced by differentiating oligodendrocyte progenitor cells (OPCs) that try to replace dying oligodendrocytes but fail to efficiently do so. MCT1 protein expression is reduced as disease progresses and we hypothesize that OPCs might also fail to initiate MCT1 protein expression as they differentiate

Objective: The goal of the current study is to enhance our insight into the ability of OPCs to differentiate into MCT1 expressing oligodendrocytes in the mouse SOD1G93A disease pathogenesis and to interfere with this process in order to enhance OPC maturation. These studies will help explore OPC and oligodendroglial dysfunction in ALS

Methods: Lineage trace mapping experiments were performed using PDGFRaCreER, RosaYFP mice and MCT1 reporter mice. Changes in LRP1 expression were detected using standard immunofluorescence techniques.

Results: In order to assess the ability of newly generated oligodendrocytes to turn on MCT1 reporter expression, we performed *in vivo* fate map analysis of oligodendrocyte progenitor cells (OPCs) at the early disease stage (P60-P90) in SOD1G93A mice. In spinal cord gray matter of control animals (n=8), we found significantly more newly generated oligodendrocytes that have turned on MCT1 reporter expression as compared to SOD1G93A mice (n=5-8)($p<0.01$). This suggests that already at the pre-symptomatic disease stage, the trophic supportive function of oligodendrocytes is impaired due to failure of newly generated oligodendrocytes to differentiate into MCT1 reporter expressing cells. One particular mechanism that interferes with OPC to oligodendrocyte maturation is mediated by the low density lipoprotein receptor-related protein 1 (LRP1) (A. Gaultier, their unpublished observations). Using antibodies directed against LRP1, we found LRP1 to be significantly upregulated in the spinal cord gray matter of presymptomatic SOD1G93A mice and *post mortem* ALS patient tissue. LRP1 expression was

detected in astrocytes and OPCs and less so in microglia as disease progresses in SOD1G93A mice.

Discussion: We found that even in the early disease stage of SOD1G93A mice, the majority of the newly generated oligodendrocytes do not fully mature and fail to provide trophic support to motor neurons. Ongoing experiments are aimed to improve OPC maturation and MCT1 expression in oligodendrocytes by modulation of the OPC differentiation through LRP1. Overall, these studies provide a core pathway for oligodendroglial-based injury to motor neurons and a mechanism for disease initiation and propagation.

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P293 TARGETING MICROTUBULES TO IMPROVE OUTCOMES IN ALS

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Keywords: cytoskeleton, motor neurons, SOD1

Background: The degeneration of axonal processes in neurodegenerative diseases has suggested that stabilisation of the axon may be an attractive target for neurodegenerative therapeutics. The microtubule stabiliser EpothiloneD (EpoD) has showed promise in ameliorating pathophysiology in a range of neurodegenerative disease models, however its effect on ALS pathology is yet to be elucidated.

Objectives: To determine the effect of EpoD on disease progression in the mSOD1^{G93A} transgenic (Tg) model using histological and behavioral methods.

Methods: mSOD1^{G93A} mice and wild-type (Wt) controls were treated with EpoD (2.0mg/kg) or DMSO vehicle control every 5 days, from 50 days to ethical end stage (158 $\pm$ 8 days) (n=46). Motor behaviour, clinical phenotype and survival were evaluated until end stage, as well as histopathological outcomes at 10 weeks and 20 weeks, by utilising YFP-mSOD1^{G93A} crossed mice (n=18).

Results: EpoD treatment delayed disease onset, as measured by hindlimb grip strength, in mSOD1^{G93A} mice by seven days, compared to vehicle treated mSOD1^{G93A} controls. Similarly, at 10 weeks EpoD treatment resulted in less pathology, with significantly ($p<0.01$) more spinal MNs (8.971 $\pm$ 1.291) and a higher proportion of intact distal axons (84.75% $\pm$ 1.22%) in treated mSOD1^{G93A} mice, compared to vehicle controls (5.095 $\pm$ 0.5732 (MNs); 66.30% $\pm$ 1.22% (proportion intact axons)). However, by 20 weeks, both cellular and axonal pathology levels in the EpoD treated mice mSOD1^{G93A} were similar to vehicle treated animals. In contrast to the grip strength results, EpoD treatment resulted in significantly more rapid decline in clinical performance, rotarod performance and weight loss. At end stage, EpoD treated mice had significantly reduced rotarod performance ($p<0.05$), clinical scores ($p<0.01$) and mean number of spinal MNs (2.800 $\pm$ 0.3177 EpoD, 1.684 $\pm$ 0.1348 vehicle, $p<0.05$), in comparison to vehicle treated mSOD1^{G93A} mice. Indeed, EpoD administration significantly decreased the median days of survival (156 days), compared to vehicle treated controls (165 days) ($p<0.01$),

Discussion and conclusions: The heterogeneous nature of ALS pathology will likely necessitate a combination of therapeutics to positively modify or alleviate the disease phenotype. Findings from this study highlight the relevance of microtubule stabilisation as a potential therapeutic intervention for the treatment of ALS. Our results demonstrate that EpoD is a disease-modifying agent in the mSOD1^{G93A} mouse model; EpoD had both positive and negative effects on specific aspects of motor function, however it had a negative influence on survival. EpoD administration was beneficial in early disease stages, when mice began to show signs of disease onset. However, EpoD treatment was not beneficial during mid to late disease stages. Future studies aim to investigate how the positive effects of EpoD can be exploited by varying dosage and timing of administration relative to disease progression.

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P294 ADAMTS-4 IS DETRIMENTAL IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: ADAMTS-4, perineuronal nets, neurodegeneration

A disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) proteoglycanases are specialized in the degradation of chondroitin sulfate proteoglycans and participate in mechanisms mediating neuroplasticity. Despite the beneficial effect of ADAMTS-4 on neurorepair after spinal cord injury, the functions of ADAMTS proteoglycanases in other CNS disease states have not been investigated. Therefore, we investigated the expression, effects and associated mechanisms of ADAMTS-4 during amyotrophic lateral sclerosis (ALS) in the SOD1^{G93A} mouse model. ADAMTS-4 expression and activity were reduced in the spinal cord of SOD1^{G93A} mice at disease end-stage when compared to WT littermates. To counteract the loss of ADAMTS-4, SOD1^{G93A} and WT mice were treated with saline or a recombinant

ADAMTS-4 before symptom onset. Surprisingly, ADAMTS-4 worsened the prognosis of $SOD1^{G93A}$ mice by accelerating clinical signs of neuromuscular dysfunctions. The worsened prognosis of ADAMTS-4-treated $SOD1^{G93A}$ mice was accompanied by increased degradation of perineuronal nets enwrapping motoneurons and increased motoneuron degeneration in the lumbar spinal cord. Motoneurons of ADAMTS-4-treated $SOD1^{G93A}$ mice were more vulnerable to degeneration due to the loss of their extracellular matrix envelopes. The decrease of neurotrophic factor production found to be induced by ADAMTS-4 *in vitro* may also contribute *in vivo* to create a hostile environment for motoneuron especially when devoid of a net. This study is the first to show that ADAMTS-4 treatment is deleterious in a mouse model of ALS by promoting neurodegeneration. Therapeutic approaches aimed at decreasing ADAMTS-4 expression/activity may therefore represent interesting targets to slow down neurodegeneration in chronic CNS diseases.

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P295 SPECIFIC TRANSDUCTION OF CORTICOSPINAL MOTOR NEURONS BY AAV2 UPON DIRECT MOTOR CORTEX INJECTION

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Keywords: upper motor neurons, AAV, corticospinal motor neurons

Background: Corticospinal motor neuron (CSMN) degeneration is a hallmark in many motor neuron diseases and it is important to improve their health to bring long-term and effective treatment strategies. Adeno-associated virus (AAV) mediated gene delivery approaches offer many advantages due to robust gene expression in CNS and lack of immunoreactivity in humans.

Objective: Targeting only a distinct neuron population within the complex structure of the brain is challenging. Here we explore the use of AAV for selective transduction of CSMN, without affecting other neurons or circuitries in the brain. In this study, seven different AAV serotypes that harbor the eGFP gene were tested for their ability to transduce CSMN upon direct injection into the layer V of the motor cortex.

Methods: We used seven AAV serotypes (AAV2-1, AAV2-2, AAV2-5, AAV2-6, AAV2-7, AAV2-8, and AAV2-9) that harbor the eGFP gene under the control of the CMV promoter. AAVs were injected directly into the motor cortex in conjunction with retrograde labeling with red fluorescent microspheres to marks CSMN in the motor cortex and to investigate the specific tropism for CSMN. In addition to co-labeling with red fluorescent microspheres, Ctip2 co-localization was used to confirm CSMN transduction.

Results: Large pyramidal neurons in layer V showed higher tropism for AAV2-2. To increase the selective transduction of CSMN by AAV, we tested capsid proteins that are engineered, and use different promoters for eGFP expression. Our results suggest that the choice of the promoter is critically important to enhance selective gene expression in CSMN, and that effective genetic modulation of CSMN is possible via direct motor cortex injection.

Discussion and conclusion: Identification of AAV serotypes that primarily transduce both healthy and diseased CSMN is critically important to deliver the genes of interest to the neurons in need without affecting other neurons or circuitries in the cerebral cortex.

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P296 MOTOR NEURONAL TARGETING BY SELF-COMPLEMENTARY AAV9 VIA INTRA-CSF DELIVERY IN NONHUMAN PRIMATE FOR MOTOR NEURON DISEASES

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Keywords: AAV, non human primate, intra-cerebrospinal fluid

Adeno-associated viral (AAV) gene therapy has shown hope for clinical treatment of neurodegenerative

diseases, however one limit of direct brain injection of AAV vectors is the lack of widespread gene expression in the central nervous system (CNS). Therefore, diffuse vector delivery covering large CNS areas remains a critical issue for future gene therapy trials. The aim of this study was to evaluate the feasibility, the efficiency and the safety of intra-cerebrospinal fluid (CSF) gene delivery to CNS of non-human primates using AAV vectors and GFP as reporter gene. We designed a safety surgical procedure for delivering AAV vector into the cerebrospinal fluid of non human primates to target CNS and investigated the CNS transduction pattern of AAV9 serotype known for its strong neurotropism, after intrathecal (lumbar puncture) or intracerebroventricular delivery. We used these two medical procedures that are authorized in human medicine in contrast to intracisternal administration.

All intraCSF administrations were performed under radioscopic real-time examination. The monitoring of the CSF pressure, the magnetic resonance imaging follow-up and the neurobehavioral and neurological examinations confirm the safety of the procedure.

A single intrathecal administration of AAV9 led to efficient and widespread transduction of spinal motor neurons from cervical to lumbar regions. Remarkably, a large proportion of motor neurons from the cervical to the lumbar spinal cord was also transduced after AAV9 intracerebroventricular delivery. In addition, this latter strategy led to a widespread gene expression in brain neurons including pyramidal neurons that was mainly located *i)* around intraparenchymatous vessels, and *ii)* closed to the lateral ventricles and meninges. Ependymal cells also expressed the GFP. Finally, GFP expression was observed in heart, skeletal muscle and liver indicating that CSF delivery of AAV vectors does not restrict gene transfer exclusively to the CNS. We have also shown a significant amount of viral particles in the serum at one hour post-injection probably secondary to reaborption of CSF into venous sinus blood. Even if immune investigations showed an humoral and cellular immune response against the GFP transgene, GFP expression was still detectable at each level of spinal cord in all non human primates. We demonstrated that lumbar intrathecal and intracerebroventricular administrations of AAV vectors are safe and reproducible in non-human primates, and therefore suitable for clinical trials.

Results of this study could have considerable impact on defining clinical trials applied to human neurodegenerative diseases.

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P297 CHRONIC INHIBITORY EFFECT OF RILUZOLE ON TROPHIC FACTOR PRODUCTION

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Keywords: Riluzole, glial cells, trophic factors

Riluzole, the only FDA approved drug for the treatment of amyotrophic lateral sclerosis (ALS), affords moderate protection to ALS patients by an unknown mechanism. In the presence of riluzole, astrocytes increase production of trophic factors that protect motor neurons. We hypothesize that stimulation of trophic factor production by motor neuron associated cells contribute to riluzole's protective effect in ALS. We investigated the effects of media conditioned by astrocytes and Schwann cells acutely or chronically treated with riluzole on trophic factor-deprived motor neuron survival. Conditioned media from astrocytes and Schwann cells acutely treated with riluzole protected motor neurons from trophic factor deprivation-induced cell death. Motor neuron protection was prevented by co-incubation with cardiotrophin-1 (CT-1) neutralizing antibodies. In contrast, conditioned media from astrocytes and Schwann cells chronically treated with riluzole was not protective ($p<0.05$). Acute and chronic treatment of mice with riluzole showed opposite effects on trophic factor production in spinal cord, sciatic nerve and brain. There was an increase in the production of CT-1 and glial derived neurotrophic factor (GDNF) in the spinal cord and CT-1 in the sciatic nerve during the first days of treatment with riluzole, but levels dropped significantly after chronic treatment with the drug. Similar results were observed in brain for CT-1 and brain derived neurotrophic factor while GDNF levels were unaffected following riluzole treatment ($p<0.05$). Our results demonstrate that the therapeutic effects of riluzole may regulate cell specific protein synthesis. Discontinuous riluzole administration to favor the acute effects on trophic factor production may improve patient survival.

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P298 ULTRA-HIGH DOSE ADMINISTRATION OF METHYLCOBALAMIN DELAYED SYMPTOMATIC AND NEUROPATHOLOGICAL FINDINGS IN WOBBLER MOUSE MOTOR NEURON DISEASE

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Keywords: methylcobalamin, therapeutic potential, wobbler mouse

Background: A previous double-blind controlled cross-over trial of ultra-high dose methylcobalamin suggested an increase of averaged compound muscle action potential amplitudes in amyotrophic lateral sclerosis (ALS) patients. Currently, this larger-scale randomized double-blind trial is under analysis in Japanese patients with ALS (Clinicaltrial.gov NCT00444613). High doses of methylcobalamin administration had therapeutic benefits in patients with peripheral neuropathy and rodent models of peripheral neuropathy or peripheral nerve injury.

Objectives: Recent studies suggested several similar profiles of clinico-phenotypic, molecular and neuropathological changes between the wobbler mouse and ALS patients. We evaluated whether methylcobalamin, a vitamin B12 analogue, can ameliorate motor dysfunction and neuropathological findings in this ALS-like animal model.

Methods: Immediately after the symptomatic onset of tremulous body at the postnatal age of 3-4 weeks, affected mice received methylcobalamin (3 mg/kg/day or 30 mg/kg/day) or vehicle for 4 weeks by intraperitoneal administration in a blind fashion. Motor symptoms of the forelimb deformity and the forelimb strength were assessed by the quantitative scale and the special dynamometer. After treatment, neuropathological changes in the biceps muscle, the musculocutaneous nerve and the cervical cord (C5-6 segments) were compared among three groups (n=10/group). Vitamin B12 concentrations of the serum, the skeletal muscle and the spinal cord at one hour after the final administration of two doses methylcobalamin or vehicle were measured in three experimental groups and normal littermates without treatment (n=5/group).

Results: Higher dose of methylcobalamin administration retarded progression of forelimb muscle weakness ($P<0.01$) and forelimbs contracture ($P<0.01$) from the 3rd week post-treatment compared to vehicle. Higher dose of methylcobalamin treatment suppressed denervation muscle atrophy ($P<0.01$) and axonal degeneration in the musculocutaneous nerve ($P<0.05$) compared to vehicle. The total number of cervical motor neurons was increased approximately 20% in the higher dose methylcobalamin-treated wobbler mice compared to vehicle-treated wobbler mice, but not significant statistically. Vitamin B12 concentrations were elevated 5-6-fold in the serum, 3-5-fold in the biceps muscle and 3-4-fold in the spinal cord of higher dose methylcobalamin-treated wobbler mice compared to vehicle and untreated normal littermates.

Discussion and conclusions: The present study supported neuroprotective effects of ultra-high dose methylcobalamin on denervating muscles and degenerating motor nerves in wobbler mouse motor neuron disease. Many effective agents in animal models of ALS, including superoxide dismutase 1- transgenic mouse, failed in clinical trials of ALS patients. This experimental data provide the novel possibility that ultra-high doses methylcobalamin may have a therapeutic potential of disease-delaying modified medication in ALS patients.

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P299 KOREA FDA APPROVED ORAL YOO'S SOLUTION (YS) AS A MONO-THERAPEUTIC AGENT THAT SLOWS THE PROGRESSION OF AMYOTROPHIC LATERAL SCLEROSIS (ALS) DISEASES

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Keywords: Yoo's solution, UDCA, clinical trial

Background: ALS and bear hibernation are associated with metabolic alterations resulted from the long-term adaptation of lipids as a main energy source. However, bears return to near-normal brain and body function in the spring. This annual physiological miracle is believed to be closely related to significantly increased plasma levels of soluble ursodeoxycholic acid (UDCA) and tenfold larger bile acid pool than humans(1).

Oral Yoo's solution(YS) is the only dosage form of UDCA that delivers a therapeutic amount of UDCA to the brain and spinal cord(2). UDCA's major mechanisms of action include glucocorticoid-receptor dependent NF-kB suppression and reduction of lipid peroxidation-induced abnormalities. Oral s-UDCA treatment mainly decreased the expression of genes involved in de novo lipogenesis among the components of lipid homeostasis.

Objectives: The therapeutic efficacy and safety of YS were evaluated by a phase 3 clinical trial. This trial was based on the observed phenomenon of bear hibernation and positive results of preclinical trials with YS(3).

Methods: In a double-blind, randomized, placebo-controlled, crossover clinical trial, 63 ALS patients were randomized to receive YS (3.5 g UDCA/140mL/day) or placebo for 3 months and then crossed over from one treatment arm to the other for 3 months after a wash-out period of 1 month. The primary outcome measure was the rate of progression, measured by the Appel ALS rating scale (AALSRS), i.e., the slope of AALSRS based on Per-Protocol dataset.

Results: The 16 patients who completed the entire trial protocol showed a 42% relative slowing of rate of deterioration. The AALSRS slope was 1.63 points/month in these patients (2.24 vs. 3.88, 95% CI for difference 0.6-2.68, $p=0.004$). In 53 patients who completed either the first or second period of the study, the AALSRS slope was 1.17 points/month (2.3 vs. 3.47, 95% CI for difference 0.08-2.26, $p=0.037$).

Discussion and conclusions: Oral YS therapy had a beneficial effect on the rate of functional decline as assessed by the AALSRS. YS was well-tolerated by patients(4).

Oral YS was marketed in Korea and Japan under Korean FDA approval as a mono-therapeutic agent that slows the progression of ALS diseases. To date hundreds of ALS patients have taken oral YS.

In Japanese patient's voluntary self-reports, 36% demonstrated the partial recovery of lost neurological function, 50% showed delayed disease progression, and 14%

demonstrated diseases progression after taking YS for about 10 months.

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P300 TONGUE STRENGTH TRAINING ACCELERATES TONGUE MOTILITY DEFICITS IN THE SOD1-G93A RAT MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: exercise, bulbar, tongue

Background: The use of exercise to maintain muscle function in Amyotrophic Lateral Sclerosis (ALS) is controversial. Recent preclinical studies suggest that moderate exercise may be beneficial in ALS, while intensive exercise may be detrimental. The effects of exercise on bulbar motor function have not been studied, however. We have measured bulbar motor deficits in the SOD1-G93A mouse and rat models of ALS (1-3). We have also found that tongue force training can affect neuromuscular junction (NMJ) proteins and motor cortical excitability in rats (4,5).

Objectives: The objective of this study was to determine the effects of tongue force training on bulbar motor function, NMJ denervation, and other measures of disease progression in SOD1-G93A rats.

Methods: We used female SOD1-G93A rats and age-matched female wild-type controls. Half of each group underwent afternoon tongue force training sessions (force requirement = 10 g), while all rats were tested under minimal force conditions (force requirement = 1 g) in the mornings. Tongue force, tongue motility, number of licks per session, forelimb grip force, body weight, and survival were measured.

Results: Tongue force did not differ between the SOD1-G93A rats and healthy controls during the testing sessions, nor was it affected by training. Surprisingly, tongue motility deficits emerged sooner and were greater in the tongue force-trained SOD1-G93A rats. This effect extended to the number of licks per session in affected rats. Forelimb grip force deficits emerged earlier in the trained than untrained groups compared to their respective wildtype controls. Body weight loss, survival, and denervation of the genioglossus (tongue protruder muscle) did not differ between the trained and untrained SOD1-G93A rats.

Discussion and conclusions: The results indicate a deleterious effect of tongue force training on bulbar motor function in female SOD1-G93A rats. The facts that tongue force and neuromuscular junction innervation of the tongue were not affected suggest that factors other than lower motor neuron integrity likely accounted for this effect.

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P301 PRELIMINARY INVESTIGATION OF SAFETY AND EFFICACY OF FASUDIL IN SUBJECTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: fasudil, clinical trial,open-label

Background: Fasudil hydrochloride, a potent ROCK inhibitor, is widely used to prevent vasospasm in subarachnoid hemorrhage and pulmonary arterial hypertension. In a previous report, fasudil was demonstrated to delay disease onset, prolong survival time and reduce the loss of motor neurons in $SOD1^{G93A}$ mice. These results suggest that fasudil has potential as a treatment for amyotrophic lateral sclerosis.

Objective: To determine whether fasudil is effective and safe in treating patients with amyotrophic lateral sclerosis.

Methods: It was an open-label, single-dose, phase II study. The key entry criteria included: Clinical diagnosis of laboratory-supported probable, probable, or definite ALS; onset of disease between 3 and 36 months; FVC >60%; ALSFRS-R≥30 with respiratory items ≥10; decline of ALSFRS-R in the last 3 months before enrollment 1-8 and taking *riluzole*. All the patients were treated with fasudil for 14 days (30 mg twice a day,

intravenous) and repeated the treatment 3 months later. They were screened every 3 months since the first dose. The primary and secondary outcome measures were the slope of decline of ALSFRS-R score and survival time. Safety assessments were conducted throughout the trial.

Results: Ten patients were enrolled in the study and 9 completed the treatment. During the six-month follow-up, no severe treatment-related adverse events were observed, indicating short-term treatment safety. Compared with 18 matched controls, the declines of ALSFRS-R during the first three months in fasudil group had a trend of slowing down without significant difference (p=0.263). In the second three months, the change of ALSFRS-R was significantly greater in fasudil group than control (p=0.008). During the follow-up period, the survival time of two groups seems no difference.

Conclusions: This first pilot study in patients with ALS showed that the fasudil treatment is safe, but didn't appear to effectively modulate disease progression in six-month observation period. However, the findings from this small-scale trial cannot be considered conclusive. A long-term follow-up, larger randomized, placebo-controlled study is required.

Trial registration: ClinicalTrial.gov (ClinicalTrial.gov Identifier: NCT01935518)

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P302 LONG-TERM USE OF RILUZOLE COULD IMPROVE THE PROGNOSIS OF SPORADIC AMYOTROPHIC LATERAL SCLEROSIS PATIENTS IN CHINA

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Keywords: cumulative defined daily dose (cDDD), long-term, riluzole

Objective: To investigate the association between the use of riluzole and the prognosis of sporadic ALS patients in China.

Methods: All patients referred to our ALS center between 2007 and 2013 were followed up every 3 months till January, 2015. The cumulative defined daily dose (cDDD) of riluzole was estimated from the medical record of each patient. Survival and tracheotomy were predefined as primary outcome measures. The association between use of riluzole and survival was analyzed using the Kaplan-Meier method and Cox regression analysis. In order to analyze the relationship between dose of riluzole and survival, the riluzole group was then divided into three subgroups: low cDDD subgroup (25% lowest cDDD), medium cDDD group, and high cDDD group (25% highest cDDD). The difference between subgroups was also analyzed.

Results: Of the 1,540 ALS patients, 415 (26.9%) used riluzole and others (1,125, 73.1%) did not. In the riluzole group, the median cDDD was 2,800mg. Compared with the control group, the age at onset was older (control vs. riluzole=49.5 yrs vs. 51.1 yrs, p=0.016) and the diagnostic delay time from symptom onset was shorter (control vs. riluzole=15 months vs. 11 months, p<0.0005 in the riluzole group. BMI of the riluzole group was higher (control vs. riluzole=23.3 kg/m^2 vs. 22.63 kg/m^2, p=0.001). The score of both FRS and FRS-R at first visit was higher in the riluzole group (FRS: control vs. riluzole=32 vs.34, p<0.0005; FRS-R: control vs. riluzole=40 vs.42, p<0.0005). The difference of age at onset, diagnostic delay, BMI, FRS score and FRS-R score between the three subgroups in the riluzole group was not significant. In the Kaplan-Meier analysis, although the median survival time of the control group and the riluzole group did not show significant difference (control vs. riluzole=64 months vs. 67 months, p=0.780), the prognosis of the high cDDD group (cDDD≥8,400mg) was better than other groups (control vs. high cDDD group, p=0.001; low cDDD group (cDDD<2,800mg vs. high cDDD group, p=0.001; medium cDDD group vs. high cDDD group, p<0.0005. In the multivariate COX regression model, after adjusted for factors of age at onset, gender, diagnostic delay, BMI, residence, phenotype, Airlie House diagnostic category at presentation, contact history of pesticides and harmful gas, history of smoking and alcohol abuse and FRS-R, the high cDDD group showed a significantly better prognosis than other groups (HR (95%CI) 0.494(0.324-0.754), p=0.001).

Conclusions: Older patients or patients who had higher BMI, shorter diagnostic delay time, higher score of FRS or FRS-R were more prone to use riluzole. Long-term (cDDD≥8,400mg) use of riluzole was associated with a better prognosis for ALS patients, while short-term use had little effect on survival.

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P303 A PHASE 1 TRIAL OF VM202 AS A TREATMENT FOR ALS: SAFETY DATA AND A POSSIBLE EFFICACY SIGNAL

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Keywords: Hepatocyte Growth Factor, Phase 1 Trial

Background: Hepatocyte growth factor (HGF) is a multi-functional mesenchyme-derived cytokine. Although largely thought of as an angiogenic agent, HGF has recently been identified as a neurotrophic factor. HGF and its cognate receptor (the c-MET receptor) are expressed in the peripheral nervous system as well as in various regions of the brain and spinal cord. W. Sun *et al*. (2002) found that locally sustained HGF production in neural tissue in SOD^{G93A} mice (Transgenic ALS model) alleviated the symptoms of Amyotrophic Lateral Sclerosis (ALS) through direct neurotrophic activities on motor neurons and indirect activities on glial cells. VM202, a plasmid DNA that expresses 2 isoforms of HGF, may elicit neuro-protective effects that could benefit patients with ALS.

Objective: We report interim results from a phase 1/2, open-label clinical trial to assess safety, tolerability, and preliminary efficacy of VM202 by intramuscular injections in patients with ALS. (clinicaltrials.gov #NCT02039401)

Methods: In this study, 18 subjects were treated with a total of 64 mg of VM202. The injections alternated between the upper and lower limbs on Day 0, 7, 14, and 21. Adverse events were evaluated according to severity and to their relationship with the study drug and injection procedure. Preliminary efficacy results were assessed via ALS Functional Rating Scale-Revised (ALSFRS-R), forced vital capacity, muscle strength, muscle circumference, and dynamometry. Subjects were followed for 9 months.

Results: At the time of abstract submission, 18 subjects have been enrolled with 10 subjects completing the study. The median age was 54.5 with 15 males and 3 females. The subjects had an average of 13 months since their diagnosis of the disease. In the interim analysis, 64 mg of VM202 was shown to be well tolerated, with no reported adverse events related to drug and few mild and moderate adverse events related to the injection procedure. There was no serious adverse event attributable to VM202. All subjects were alive at interim analysis. Mean ALSFRS-R score at baseline was 38.7±4.4, and their monthly changes in ALSFRS-R after VM202 administration were 1.0, 0.44, 0.79, and 1.8 at month 1, 3, 6, and 9, respectively.

Discussion and conclusion: Multiple intramuscular injections at different site appear safe in ALS subjects at the doses tested, and further clinical trials to assess efficacy and safety should be considered. VM202 appears to stabilize the clinical condition between 3 to 6 month periods, suggesting that additional dosing may be required 3 to 6 months after initiation.

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P304 TRPV1 AND TRPA1 ACTIVATORS REDUCE MUSCLE CRAMPING: A POTENTIAL NEW TREATMENT FOR ALS SYMPTOMS

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Keywords: cramps, spasticity, TRP

Background: Muscle cramps and spasticity can be painful and debilitating for patients suffering from ALS and other neurological diseases. A Boston-based biotechnology company, Flex Pharma, Inc., is developing innovative treatments for neuromuscular conditions/muscle cramps and spasms. Recent studies have demonstrated that hyperexcitability of α-motor neurons in the spinal cord is likely the underlying cause of cramps and spasticity.

Objectives: Based on a general property of neuronal circuits, whereby strong excitatory input increases inhibitory tone and reduces responsiveness to excitation, we hypothesized that transient receptor potential channel (TRP) activation could yield sufficient excitatory input to dampen motor neuron hyperexcitability.

Methods: To test this hypothesis, we conducted human studies to assess the effectiveness of TRP activation in inhibiting electrically-induced cramps of the foot. Cramp of the flexor hallucis brevis muscle was elicited through external stimulation of the abductor hallucis brevis muscle and medial plantar nerve. The intensity and duration of the cramp was recorded by EMG for each subject before and after treatment in time course studies. Each subject served as their own control.

Results: An oral solution containing TRP activators was shown to prevent cramps within minutes of ingestion, lasting up to 6-8 hours. The aggregated results from three independent, randomized, blinded clinical studies showed a significant reduction in cramp intensity by 3-fold ($p<0.0001$). Review of EMG output showed that cramps were reproducibly elicited with little intra-subject variation for threshold settings and cramp profile. However, EMG patterns varied considerably between subjects. Cramp profiles (area under the curve for cramp intensity and duration) fell under several sub-types: 1) low intensity but sustained for several minutes on EMG, 2) high intensity with a rapid return to baseline, 3) delayed onset, 4) high intensity and sustained, and 5) multi-phasic sustained. Most subjects fell into subtype 1. There was no difference between the threshold settings to induce cramp and cramp profile. The Flex proprietary formulation was effective at reducing cramp intensity across all cramp subtypes.

Conclusions: These results suggest that TRP activators may be an effective new treatment for individuals suffering from cramps and spasticity associated with ALS. As such, Flex Pharma plans to initiate a multi-center, randomized, blinded, 14-day cross-over study to investigate the effects of Flex's proprietary formulation in patients with symptoms of cramps due to ALS. Patients will be assessed for the safety and tolerability of the product as well as changes in their cramp frequency and severity. The results from pre-clinical and ongoing clinical research will be presented.

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P305 THE ALS TREATMENT PRIZE CHALLENGE – LEVERAGING THE INCENTIVE PRIZE MODEL TO ACCELERATE PRECLINICAL DRUG DEVELOPMENT IN ALS

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Keywords: SOD1, Preclinical, Crowdsourcing

Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by death of

motor neurons in the brain and spinal cord, leading to progressive paralysis and death within an average of 2-5 years from diagnosis. At present, there is no effective ALS therapy and the only FDA-approved drug for ALS, riluzole, prolongs survival by only 2-3 months. Effective disease-modifying therapies for ALS are urgently needed.

Objectives: In order to increase the number of new candidate therapies entering the ALS drug development pipeline, and to attract new minds to the field, Prize4life launched the $1M Avi Kremer ALS Treatment Prize4Life (ALS TP) challenge - a $1 million award for a therapy that reliably and effectively extends the life of the most widely used ALS mouse model by 25%. The challenge aimed to identify a preclinical-stage candidate therapy that could attract the attention of industry partners with the necessary resources to move the therapy into the clinic.

Methods: With the support of the Prize4Life Scientific Advisory Board, the ALS TP was first launched in 2008. The challenge required testing in the G93A-SOD1 mouse model, and specified requirements for the experimental design and complete data package. In order to support participating teams, Prize4Life partnered with the Jackson Laboratory (TJL), a leading provider of mouse models for research, to establish an ALS mouse colony to provide quality-controlled mice free of charge. In addition, in 2008 Prize4Life and TJL published a guidance manual of best practice recommendations for scientific studies in the ALS mouse models, which has been widely used by ALS researchers worldwide.

Results: The first launch of the ALS TP closed in 2010. 34 teams actively participated, of which 46% were new to the field of ALS, and 13 teams submitted a solution. Participants tested a wide range of therapeutics, ranging from small molecules (44%), to protein biologics (44%) to cell-based approaches (11%). With no winner, the prize was relaunched in 2012 and closed in 2015. The prize attracted 35 teams of which 40% were industry-based and 60% were academic teams. The companion mouse colony supported preclinical testing of over 40 new drug candidates. At the ALS MND meeting, we will present the leading submissions and the profile of the candidate therapies tested.

Discussion and conclusions: The ALS Treatment Prize, with companion resources, has been an effective mechanism to attract new researchers to the field of ALS and to foster rigorous preclinical testing of a wide range of new candidate ALS therapies. These results demonstrate the value of incentive challenges in attracting attention to unmet needs and spurring innovative, rigorous biomedical research.

Acknowledgements: We acknowledge the Jackson Laboratories for the partnership surrounding the SOD1 Mouse Colony, our generous donors, and all participating teams.

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P306 CONNECTING ALS PATIENTS AND FUTURE CLINICAL TRIALS BY TRICALS: A WEB-BASED INTERNATIONAL PATIENT REGISTRATION PLATFORM

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Keywords: clinical trials, therapeutic strategies, web-based registration

Background: Without a suitable treatment for amyotrophic lateral sclerosis (ALS), disease progression will inevitably lead to paralysis of skeletal muscles, respiratory failure and eventually death. New clinical trials are necessary to investigate promising compounds and to discover more effective therapies for patients suffering from ALS. To reliably assess the effect of a potential new treatment, large randomized placebo-controlled clinical trials are needed. Due to the relative rarity of the disease, it is for clinical trials a time-consuming and costly process to gather large cohorts of ALS patients. Not only does this lead to a waste of resources but this also delays the development of new potential treatments. Therefore, there is a need that possible participants with ALS are registered centrally so that future clinical trials can quickly assemble a large cohort of patients.

Objectives: The aim of this project is to create a web-based international patient registry that speeds up the development of clinical trials for ALS.

Methods: A web-based international patient registry was created where patients with ALS can register themselves. Patients are asked to provide essential data about their disease progression (ie repeated ALSFRS-R scores, lung capacity, details about symptom onset and diagnosis). This creates the opportunity to directly contact the patients and provide them with information about novel therapeutic trials. Researchers can quickly assess and assemble a cohort of possible participants for their clinical trial. By connecting the registry with ALS centers and affiliated academic centers, several unique services can be provided to unaffiliated researchers or pharmaceutical companies such as design and development of clinical research protocols, project coordination, data management and bio-statistical data analysis.

Results: The treatment research institute for the cure of ALS (TRICALS) has since its launch mid 2014, more than 400 registered ALS patients in the Netherlands alone. Almost 60% of the registered patients actively provide and update their clinical data. In May 2015, the first TRICALS initiated clinical trial has started.

Conclusions: The TRICALS registry successfully connects ALS patients, ALS centers, academic centers and pharmaceutical companies. This creates the unique possibility to quickly select and assemble large cohorts of ALS patients willing to participate in clinical trials. Moreover, TRICALS can provide unique services for accurate and reliable clinical trial designs, analysis and results.

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P307 THE ALSUNTANGLED TABLE OF EVIDENCE

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Keywords: Alternative, Off-label, Supplements

Background: Patients with ALS (PALS) often consider alternative or off-label treatments (AOTs) they read about on the Internet. Information about AOTs can range from absent to flawed to grossly inaccurate. In 2009, the North American ALS Research Group started ALSUntangled to scientifically review AOTs and allow PALS to make more informed decisions about them. Our review team now consists of 95 clinicians and scientists from 10 different countries. To date we have received requests to investigate more than 160 different AOTs, and have completed 28 reviews which are available free on our website (www.alsuntangled.com). Recently, to make our reviews even more objective, to facilitate updating them when new information arises, and to make it easier for PALS to prioritize AOTs based on their own individual values and preferences, we decided to construct a Table of Evidence.

Methods: We found no precedent for grading the types of evidence we review, so we used a "crowd sourcing" approach to create the Table. One ALSUntangled reviewer (RB) constructed a first draft. The rest of the team then suggested edits via emails over 2 months. Finally a subset of reviewers met in person and validated the utility of the Table by attempting to convert the evidence from all prior ALSUntangled reviews into this.

Results: Five iterations of the Table occurred. Reviewers ultimately selected the following categories of evidence for the Table: Mechanism, Pre-Clinical Data, Anecdotal Data, Trials, and Risks. We decided on an ordinal, rather than numeric grading system within each category. Specific cutoffs for achieving grades of A, B, C, D, F and U were developed. During the in person meeting, information from all prior ALSUntangled reviews was translatable to the Table, consensus between reviewers was easily obtained, and new information could be quickly incorporated into the Table to update a review.

Conclusions: The ALSUntangled Table of Evidence facilitates simple, objective, reliable and timely reviews of AOTs, and should make it easier for PALS to prioritize AOT's based on their own individual values and preferences.

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