Abstracts from Theme 12

Scientific Work in Progress

Clinical Work in Progress and Care Practice

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THEME 12A SCIENTIFIC WORK IN PROGRESS

SW1 WHOLE EXOME SEQUENCING OF ALS CASES AND CONTROL: A PILOT STUDY

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Keywords: whole exome sequencing

Background: Genetic factors are a major cause of ALS even in apparently sporadic cases. Currently, the known ALS genes explain a small proportion of sporadic cases. Identification of additional risk loci for ALS will contribute to understanding of the aetiology of ALS

Objectives: To perform whole exome sequencing on 116 ALS Caucasian cases who do not carry known ALS mutations and 58 controls.

Methods: Briefly, barcoded paired-end (PE) genomic libraries were constructed from blood-derived DNA using Illumina TruSeq DNA Sample Prep Kits. Sets of six PE libraries (4 cases, 2 controls) were pooled prior to exome capture using NimbleGen's SeqCap EZ Human Exome Library v2.0. This kit uses 2.1 million probes to pull-down ~35Mb of exonic sequence spanning ~20,000 genes. Sequencing will be performed on our in-house HiSeq 2000 sequencer, with one pool of six exome libraries per v3 flow cell lane (ie 29 lanes for 174 samples). Current output on v3 flow cells is ≥36Gb/lane with ~80% on-target reads. This equates to ~5 GB of mapped sequence per person, median coverage of ~100-fold and >20-fold coverage across ~80% of the exome.

Results: WES is currently ongoing.

Acknowledgments: MND Research Australia and the National Health and Medical Research Council

SW2 THE ROLE OF SOMATIC MUTATION IN SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: sporadic, next generation sequencing, somatic

Background: Although mutations in known disease-causing genes have occasionally been identified in sporadic cases of ALS (SALS), over 80% of all ALS cases still have no known genetic aetiology. These sporadic cases may be due to environmental factors that have yet to be identified, or they may also be caused by somatic mutations in the nervous system that may be undetectable in blood.

Methods: This work has required direct collection of matched multiple tissue types at autopsy including spinal cord and blood. Following collection, genome sequencing using Next Generation Sequencing (NGS) methods of both tissue types allows comparison of the derived sequence information to determine potential somatic alterations in DNA. At present, we have collected over 20 cases and have begun to sequence and analyze the data for evidence of somatic mutations that may be causative of SALS.

We are continuing to collect these rare tissues as they become available for future sequencing projects and we welcome anatomical gifts and collaborators to supplement our study. Ultimately, we will synergistically combine our sequencing data with data being generated by other ALS genome sequencing projects.

SW3 GENOME-WIDE ASSOCIATION STUDY OF RARE VARIANTS IN ALS

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Keywords: Genome-wide association study, rare variants, genetics

Background: Following the common variants/common disease hypothesis, genome-wide association studies of ALS have mostly investigated variants with a minor allele frequency (MAF) > 5%. Until now, GWAS of common variants have identified three susceptibility loci for ALS (*C9orf72*, *UNC13A*, and a locus on chromosome 17q11.2). However, an important part of the genetic contribution to ALS susceptibility remains undefined.

Objectives: Rare variants may explain the missing heritability in ALS, and we aimed to identify variants with a low MAF (< 5%) that are associated with ALS susceptibility.

Methods: We recruited balanced strata of ALS patients and unaffected controls from six European countries (The Netherlands, Germany, Belgium, Ireland, Italy, and Spain), comprising a total of 4,497 ALS cases and 3,228 controls. Samples were genotyped using the Illumina HumanExome beadchip. Genotype calling was done using the GenCall algorithm, and clustering was based on

all samples in the project with a high genotyping call rate. Per stratum, SNP marker and per-individual quality control measures were applied.

Single-variant association analyses using logistic regression, and correcting for population substructure, are performed per stratum and combined in a weighted inverse-variance meta-analysis. Additionally, we will perform gene-based analyses using genic burden methods (eg variable threshold, SKAT).

Results: After quality control, 4,312 ALS patients and 3,133 controls were available for association analysis. For common variants (MAF > 5%), the genomic inflation factor was 1.04, indicating adequate quality control.

In the single-variant meta-analysis, we did not identify any genome-wide significant ($P < 5\times10^{-8}$) hits. The lowest *P* value was for rs3849982 near *C9orf72*, one of the common variants included on the chip (odds ratio 1.22, $P = 1.41\times10^{-6}$). Additional analyses, including gene-based tests are ongoing and will be presented.

Discussion and conclusion: Our study is the first to present a large genome-wide association study of rare variants in ALS. A single-variant analysis did not identify genome-wide significant hits, while this analysis is less appropriate and underpowered for very rare variants. Additional gene-based analyses are ongoing and results will be presented when available.

SW4 MUTATION IN THE SENATAXIN GENE FOUND IN A CHINESE PATIENT AFFECTED BY LEFT LIMB WEAKNESS AND ATROPHY WITH JUVENILE ONSET

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Keywords: Chinese, senataxin gene, juvenile onset

Case Report: We report a Chinese patient with juvenile onset, characterized by progressive weakness and wasting in left leg. The 15 years old male had a sudden tearing-like pain in the left calf muscle for three months before he came to our clinic. The pain is prominent to the rear of his left leg. It occurs when he moves or when pressed on that area, disappeared while resting. His walking distance has decreased; he could only walk 1000 meters. No fever, rash, and local redness were found. One week later the pain gradually reduced and disappeared, but he experienced weakness in his left leg and his foot drops when walking upstairs. He noticed the obvious muscle atrophy in the left leg two weeks later with no significant changes since then. The weakness in the left leg is suddenly significantly more severe one month later. His walk distance decreased to 200 meters. After supporting therapy, left leg weakness gradually returned to its earlier status with no significant changes in muscle atrophy in the left leg when compared with those two months prior. The patient has good appetite and sleep well. He has lost 4kg of body weight. The patient has a history of full-term birth, normal school sports and took polio vaccine; he has no family history of limb weakness.

Neurological examinations show clear mental status, fluent language, normal cranial nerve and normal limb muscle tone. The left leg is thinner and shorter than the right leg. At prone position he can't lift the left leg. The left femoral, gastrocnemius muscle is atrophied. The strength of left thigh abduction, flexion, knee extension, foot dorsiflexion, plantar flexion, and toe dorsiflexion is grade IV; others muscle strength is grade V. Bilateral arm tendon reflexes are symmetric. Right Achilles tendon reflex is weak and left disappeared. Bilateral Hoffman sign and Babinski' sign is negative. The right Chaddock' sign is positive, the left is suspected positive. Abdominal reflex is symmetry. The deep sensory is normal and symmetric in limbs and trunk. He has steppage gait. Romberg sign is negative.

EMG showed neurogenic changes in bilateral upper and lower limbs, with normal motor and sensory nerve conduction velocity. His head, thoracic, lumbar MRI showed no abnormalities. CK is 308U/L. CSF-Pro 0.56g/L. Blood anti-lipid antibodies, antinuclear antibody, anti-neutrophil cytoplasmic antibody, anti GM1 antibody (IgG + IgM) are negative. Serum IFE (IgA + G+ M) and urinary IFE is negative. Taken together, diagnostic work-up revealed the diffuse involvement of upper and lower motor neurons which indicate ALS. Genetic analysis revealed a C1554G mutation in the senataxin gene.

SW5 CROSS-DISEASE ANALYSIS OF COMMONLY DEREGULATED GENES AND PATHWAYS IN THE MOTONEURON DISEASES SPINAL MUSCULAR ATROPHY AND AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: spinal muscular atrophy (SMA), RNA metabolism, cytoskeleton

Background: Spinal muscular atrophy (SMA) and Amyotrophic Lateral Sclerosis (ALS) are two diseases affecting motoneurons. Whereas SMA is a monogenic disease caused by deletion or mutation of the Survival of Motoneuron-1 (Smn1) gene, mutations in a broad range of genes (eg SOD1, FUS, TDP-43, C9orf72, MATR3) are responsible for familial and sporadic cases of ALS. Although genetically distinct, the two motoneuron diseases share similar pathomechanisms regarding RNA metabolism and cytoskeletal regulation of which less is

known about. Mutant FUS tends to form cytosolic aggregates that have been shown to sequester free SMN protein (1) thus mimicking a SMA phenotype. Consequently, we hypothesize that both diseases share common mechanisms with regard to altered RNA metabolism and regulation of the neuronal cytoskeleton, maturation and survival.

Objectives: SMN is a crucial factor for the cytosolic formation and nuclear shuttling of small nuclear ribonucleoprotein particles (snRNPs) which are involved in pre-mRNA splicing. FUS is also an important factor in RNA metabolism by directly binding to SMN. As many FUS mutants are diminished in cytosolic-nuclear shuttling (1), mutations lead to partial cytosolic retention of SMN and snRNPs and changed alternative splicing (2). Independent of the nuclear role of SMN, the protein is also involved in actin cytoskeleton-regulation via Rho-kinase (ROCK) pathway which is deregulated upon SMN depletion in SMA (3) as well as in neuronal differentiation and survival via ERK- and Akt-pathways in both diseases (4). Interestingly, it was previously shown that these pathways directly interact with each other in SMA (4).

Methods: Here, we aim to find commonly deregulated genes in both diseases using RNA microarray analysis and quantitative real-time PCR, which could exhibit new insight into disease progress. Moreover, we perform phospho-pathway and co-aggregation analyses to investigate regulatory effects with several cellular models (eg cells expressing wild type or mutant FUS and SOD1). Thus, we intend to gain new insights into dysregulated signalling pathways on transcriptional and translational levels in both, SMA and ALS, pathogeneses.

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SW6 IMMUNOHISTOCHEMICAL ANALYSIS OF ERBB4 IN THE SPINAL CORD OF SPORADIC ALS PATIENTS

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Keywords: ErbB4, ALS19, sporadic ALS

Background: ALS consists of 5-10 % familial ALS (FALS) with the remaining patients presenting as sporadic ALS (SALS). To date, more than 20 genes have been shown to be associated with FALS. Identification of FALS genes contributes greatly to understanding of the pathogenesis of ALS. Recently, *ERBB4* has been identified as a novel causative gene for FALS, designated as ALS19. Functional analysis of its causative mutations revealed reduced autophosphorylation of the ErbB4 proteins upon binding of its ligands, neuregulins (NRGs). This suggests that disruption of NRG-ErbB4 pathway leads to motor neuron degeneration.

Objectives: To investigate whether ErbB4 is associated with the pathogenesis of SALS by comparing the expression patterns of ErbB4 protein in SALS patients with those in normal controls.

Methods: Paraffin-embedded sections of the spinal cord obtained from autopsy specimen of 18 SALS patients and 15 normal subjects were used for this study. Immunohistochemical analysis was performed using an anti-ErbB4 antibody sc-285 (Santa Cruz Biotechnology).

Results: In the spinal cords of normal subjects, diffuse staining of ErbB4 in the cytoplasm was observed in the motor neurons in the anterior horns. In SALS patients, striking variability of expression levels was observed in the motor neurons, some of which showed severely decreased expression levels. In advanced stage, expression levels were decreased in almost all of the remaining motor neurons in some patients. Interestingly, neurons in Onuf's nuclei, which are not affected in ALS, exhibited normal staining patterns in SALS patients. In addition, expression of ErbB4 was also observed in glial cells in some of the patients, which was not observed in normal subjects.

Discussion and conclusion: This study demonstrated that expression patterns of ErbB4 in sporadic ALS were different from those in normal subjects, raising the possibility that ErbB4 might be also involved in the pathogenesis of SALS. Considering that disruption of NRG-ErbB4 pathway could be the mechanism underlying ALS19, the decreased expression of ErbB4 might accelerate the degeneration process of motor neurons. Expression of ErbB4 in the glial cells in sporadic ALS implies that ErbB4 might be also involved in the non-cell autonomous mechanism of motor neuron degeneration.

Acknowledgement: This study was sponsored by ALS Foundation, Japan ALS Association.

SW7 DEFECTIVE RECOGNITION OF ATG8/LC3 PROTEINS BY MUTANT P62/SQSTM1 IMPLICATES DYSREGULATION OF AUTOPHAGY IN ALS/FTLD

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Keywords: p62/SQSTM1, ATG8/LC3, autophagy

Background: Autophagy is an essential pathway for the degradation of damaged and aggregation-prone proteins and organelles, and dysregulation of autophagy has been proposed as a pathophysiological mechanism in ALS/FTLD. The p62/SQSTM1 protein is a cargo receptor for ubiquitin-mediated autophagy. A critical protein-protein interaction in autophagy involves recognition of lipid-anchored ATG8/LC3 proteins within the autophagosome membrane by p62/SQSTM1, mediated through the LC3-interacting region (LIR, residues 337-347) of the latter. Recently mutations affecting the SQSTM1 gene, which encodes the p62/SQSTM1 protein, have been identified in patients with ALS/FTLD and several of these mutations map within or close to this LIR sequence.

Objectives: To determine whether ALS/FTLD-mutant p62/SQSTM1 proteins are associated with defective recognition of ATG8/LC3 and by extension, dysregulation of autophagy.

Methods: We analysed the effects of several ALS/FTLD-associated mutations of p62/SQSTM1 (within or close to the LIR) on protein function focussing on the interaction with LC3B, using a combination of biochemical (pull-down assays) and biophysical techniques (mass spectrometry, nuclear magnetic resonance spectroscopy).

Results: Biochemical analyses demonstrate that selected disease-associated LIR mutations of p62/SQSTM1 result in defective recognition of LC3B. Biophysical analyses more fully define the LIR sequence of p62/SQSTM1 and place the effects of the mutations on a firm quantitative and structural footing, providing a molecular rationale of how they impact on protein function.

Discussion and conclusions: Our findings support the notion that disease aetiology in ALS/FTLD with SQSTM1 mutations involves dysregulation of autophagy, which we speculate may represent a wider mechanistic aberration that crosses over between other mutational and non-mutational causes.

Acknowledgements: RL, MSS and AG are supported by the MND Association, UK (Ref: 6095).

SW8 THE R399G MUTATION IN THE CYTOPLASMIC DYNEIN HEAVY CHAIN DISRUPTS GOLGI CISTERNAE IN HUMAN FIBROBLASTS.

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Keywords: dynein, golgin 160, golgi

Background: A neurodegenerative disorder known as spinal muscular atrophy with lower extremity predominance (SMA-LED) refers to a congenital condition presenting with lower limb wasting, and cognitive impairment in some cases. Neurogenic deterioration observed in this disease has been narrowed to autosomal dominant mutations in the heavy chain subunit of cytoplasmic dynein (DYNC1H1). Cytoplasmic dynein is a multi-subunit complex integral for multiple cellular processes including retrograde transport, microtubule stability, and Golgi positioning.

Objectives: Here we report our investigation on the impact of a missense R399G mutation, located in the homodimerisation domain of DYNC1H1, on the integrity of the Golgi apparatus.

Methods: The DYNC1H1$^{+/R399G}$ and DYNC1H1$^{R399G/R399G}$ fibroblasts were treated with nocodazole for 1 hour in order to fragment the Golgi before measuring the degree of fragmentation after nocodazole washout. Immunofluorescence investigations were subsequently carried out using poly giantin to analyse the extent of Golgi recovery. As golgin 160 has been found to be the tether between Golgi membranes and microtubule bound dynein, golgin 160 pull down was additionally conducted.

Results: Human fibroblasts were measured for the extent of Golgi fragmentation (n>870 cells) pre nocodazole treatment and post nocodazole washout at 0, 40, and 60 min time points. The Golgi was found to be more fragmented in the R399G untreated cells, correlating in a dose dependent manner to the zygosity of the mutation, with DYNC1H1$^{R399G/R399G}$ fibroblasts exhibiting statistically significant fragmentation of the Golgi in comparison to wild type cells ($p<0.01$). At the 60 min time point after nocodazole washout the DYNC1H1$^{+/+}$ cells fully reassembled Golgi cisternae. In contrast DYNC1H1 $^{+/R399G}$ and DYNC1H1$^{R399G/R399G}$ fibroblasts failed to recover after 60 min. Moreover, immunoprecipitation revealed an increased interaction between golgin 160 and the cytoplasmic dynein complex.

Discussion and conclusion: The DYNC1H1^{R399G} mutation results in both increased Golgi fragmentation as well as delayed cisternae re-assembly after nocodazole treatment. This indicates a structural aberration in the Golgi apparatus as a result of the R399G substitution mutation in DYNC1H1. Additionally the observed enhanced golgin-160 interaction with the dynein complex is likely a cellular compensatory mechanism in order to resolve dysregulated Golgi structure.

Acknowledgements: Funding provided by the Hans and Marit Rausing Scholarship and the University of Sussex, School of Life Sciences. Fibroblasts were acquired by the MRC centre for neuromuscular disease Biobank.

SW9 MAHOGUNIN RING FINGER PROTEIN 1, UBIQUITIN-PROTEIN LIGASE CONFERS NEUROPROTECTION AGAINST MISFOLDED PROTEIN AGGREGATION AND TOXICITY

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Keywords: SOD1, protein quality control, ubiquitin ligase

Background: Accumulation of misfolded proteins is involved in the pathomechanism of motor neuron disease. The precise protein quality control system by Mahogunin ring finger protein-1 (MGRN1), E3 ubiquitin protein ligase against misfolded proteins including mutant SOD1 in neurons remains obscure. In addition, elimination of MGRN1 in mice causes spongiform neurodegeneration in an age dependent manner.

Objectives: The aim of this study is to reveal the neuroprotective mechanisms of MGRN1.

Methods: Using cultured cells, interaction of MGRN1 with molecular chaperons, and MGRN1 levels under various stress condition including ER stress, autophagic inhibition were examined. Moreover, neuroprotective potential of MGRN1 against misfolded proteins was tested.

Discussion and conclusion: MGRN1 was induced under various stress conditions and interacted with Hsp70 chaperone and misfolded proteins. Moreover, MGRN1 promotes the degradation of misfolded proteins and protects cells against ER and oxidative stress. The neuroprotective potential of MGRN1 against mutant SOD1 mediated toxicities is underway.

SW10 DEVELOPMENT OF A CELLULAR TDP-43 AGGREGATION ASSAY BASED ON PROTEIN COMPLEMENTATION

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Keywords: TDP-43, aggregation, protein complementation

Background: Tar DNA-binding protein 43 (TDP-43) was shown to oligomerize/ aggregate in affected neuronal and glial cells in amyotrophic lateral sclerosis (ALS) (1, 2). Moreover, causative mutations in the TDP-43-endcoding TARDBP were identified in genetic ALS patients.

Objective: The aim of our study was to establish a cellular, quantitative TDP-43 oligomerization assay of TDP-43.

Method: For this purpose we used an innovative protein fragment complementation assay, based on non-bioluminescent N- or C-terminal Gaussia princeps luciferase fragments fused to the C-terminus of TDP-43. Oligomerization of TDP-43 leads to reassembly of the two luciferase fragments, thereby restoring luciferase activity.

Results: We were able to show that there is no intrinsic self-complementation of the luciferase fragments themselves without fusion to TDP-43. Results from our novel assay correlated with data based on size-exclusion chromatography and confirmed that mutated TDP-43 or TDP-43 in stressed cells has a higher tendency to oligomerize compared to wildtype protein under unstressed conditions (3). Thus, the TDP-43 complementation assay enables quantitative assessment of TDP-43 oliomerization in living cells in a high-throughput manner. Furthermore, we present data demonstrating that the novel assay can be used for quantitative studies on the subcellular distribution of TDP-43 oligomers, cell-to-cell transmission of TDP-43 pathology in living cells and compartment-specific uptake and transport of TDP-43 by neurons. Using microfluidic chambers we show that neurons are able to take up TDP-43 by their axonal terminals and transport it retrogradely.

Discussion and conclusion: Taken together, the new TDP-43 complementation assay represents a versatile tool to study TDP-43 biology and might help in identifying drug candidates for the treatment of ALS.

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Acknowledgement: Charcot Foundation

SW11 GLUTATHIONE MONO-ETHYL ESTER REDUCES FORMATION OF CYTOSOLIC TDP-43 AGGREGATES IN NSC-34 CELLS

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Keywords: glutathione, oxidative stress, NSC-34

Background: The pathological signature of several neurodegenerative diseases including motor neuron disease (MND) is accumulation of ubiquitinated cytosolic protein aggregates that include the nuclear TAR-DNA binding protein TDP-43. This protein regulates transcription, transport and post-transcriptional modification of mRNAs. TDP-43 pathology is triggered by abnormal processing and cytosolic aggregation of the

protein which is promoted by mutations in the TDP-43 gene and is associated with nuclear depletion of the protein. Mutant TDP-43 causes familial forms of MND, MND-like disease in animals and kills motor neurons in culture. TDP-43 is highly susceptible to changes in cellular redox status and depletion of cellular antioxidants lead to cytosolic aggregation of this protein similar to what is observed in TDP-43 proteinopathies.

In this context, the endogenous antioxidant glutathione (GSH) is central. GSH is a major endogenous antioxidant in cells, acting both to directly detoxify reactive oxygen species and as a substrate for several peroxidases. We have previously shown that GSH is depleted in cells stably expressing the hSOD1^{G93A} mutation and that disease or treatments that deplete cellular glutathione commonly promote cell death. Moreover, oxidative stress and changes in cellular GSH content are common features in human MND.

Objectives: The aim of this study was to evaluate the role of GSH in TDP-43 pathology and to determine if restoring cellular GSH prevents or reduces cytosolic aggregation of TDP-43.

Methods: Subcellular localisation of TDP-43 and phosphorylated TDP-43 were quantified using western blots and immunohistochemistry. Total, reduced and oxidised GSH (GSSG) was quantified using spectrophotometry. NSC-34 cells were transfected, using nucleofection, with the A315T TDP-43 mutation and were compared to cells transfected with a control plasmid.

Results: Wild-type/control NSC-34 cells contained 43±8 nmol GSH/mg protein and a GSH/GSSG ratio of 55±12. Treatment of wild-type NSC-34 cells with ethacrynic acid (70 µM/5h) reduced GSH content to 29±5 nmol/mg protein and the GSH/GSSG ratio to 11±3 (n=4). This treatment also resulted in cytosolic TDP-43-positive cytosolic aggregates in a majority of the cells. Co-treatment with glutathione-monoethyl ester (GME: 10 mM) prevented changes in GSH content and the occurrences of cytosolic TDP-43 aggregation (n=4). Cells expressing the A315T mutation had reduced GSH content (35±3 nmol/mg protein). The GSH/GSSG ratio in these cells was significantly reduced (8±2) indicative of oxidative stress response (n=4). In cells carrying mutant TDP-43, cytosolic inclusions were detected in 34% of the cells whereas none were found in control cells. Preliminary data suggest that GME treatment of cells expressing mutant TDP-43, restore GSH content and reduces cytosolic TDP-43 aggregation (n=2).

Discussion and conclusion: This study provides early data strongly suggesting that GME could be beneficial in treatment of TDP-43 proteinopathies.

SW12 CLEARANCE OF THE ALS-ASSOCIATED TDP-43 PROTEINOPATHIES BY THE ACTIVATION OF HEAT SHOCK RESPONSE

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Keywords: HSF-1, DNAJB2, TDP-43

Background: TDP-43 proteinopathy is a clinical phenotype observed in the affected neuronal tissues of most amyotrophic lateral sclerosis (ALS) and about 60% of frontotemporal lobar degeneration (FTLD) cases. Affected cells are characterised by the accumulation of detergent-resistant ubiquitin positive hyperphosphorylated TDP-43 aggregates in the cytoplasm.

Objectives: In this study, we established a cell model, which recapitulates TDP-43 proteinopathy and used it to investigate whether manipulation of the heat shock response (HSR) could induce the clearance of insoluble TDP-43.

Results: Under the control of heat shock factor 1 (HSF1), the HSR is a stress-inducible cell protective mechanism resulting in the increased expression of protein chaperones that refold misfolded proteins and facilitate protein trafficking and degradation. Our study demonstrates that manipulation of the HSR by expressing dominant positive HSF1 results in a dramatic reduction of insoluble and hyperphosphorylated TDP-43, coupled with enhanced cell survival. On the other hand, expression of dominant negative HSF1 exacerbates detergent resistance and hyperphosphorylation of TDP-43. As the activation of HSF1 induces a vast number of heat shock proteins (HSPs), we screened the major HSPs and most of the HSP40 proteins and identified one of the co-chaperones, DNAJB2a, as participating in this HSF1-induced TDP-43 clearance in a HSP70-dependent manner. DNAJB2a has both a J domain, allowing it to interact with HSP70, and ubiquitin interacting motifs (UIM), which allows it to engage the degradation of its client proteins. We found that the DNAJB2a-mediated TDP-43 clearance is independent of the UIM and not affected by proteasome inhibition. On the other hand, disrupting its interaction with HSP70 inhibited the DNAJB2a-mediated TDP-43 clearance, indicating the insoluble TDP-43 is refolded by HSF1-induced DNAJB2a while total levels of TDP-43 remain unchanged.

Discussion and conclusion: In summary, we showed the ALS-associated TDP-43 proteinopathies could be resolved by activating the HSF1-DNAJB2a pathway. The important aspect of this mechanism is that it does not resolve TDP-43 proteinopathies by degrading the misfolded proteins but by facilitating protein refolding. As TDP-43 is highly auto-regulated to maintain its cellular level, we believe the pathway that promotes protein refolding is much more efficient than the one enhancing degradation, and are currently looking into therapeutic intervention in this respect.

SW13 ABSTRACT WITHDRAWN

SW14 CHARACTERISATION OF NEURONS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS FROM MOTOR NEURONE DISEASE PATIENTS IDENTIFIES ALTERATIONS IN PROTEOSTATIC MECHANISMS

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Keywords: proteostasis, induced pluripotent stem cells, ubiquitin

Background: Identification of the specific mechanisms by which motor neurones degenerate and die in motor neurone disease (MND) has previously been hampered due to a lack of appropriate disease models. However the advent of induced pluripotent stem cells and rapid progress in the methods to differentiate these cells into functional motor neurones has provided a means to model both familial and sporadic MND in a dish.

Objectives: To establish induced pluripotent stem cell models from MND patients, to gain an understanding of motor neurone dysfunction in MND.

Methods: Non-integrating mRNA reprogramming methods were used to generate induced pluripotent stem cells from MND patient or control fibroblasts (n=22). Systematic characterisation of the MND-related pathology in the motor neurones from each patient was carried out by immunocytochemistry with antibodies to TDP-43, ubiquitin and a panel of motor neurone, glial and synapse markers. Neuronal function was characterised by Ca^{2+} imaging, Apotox apoptosis and cytotoxicity and ubiquitination assays.

Results: We have generated induced pluripotent stem cells lines from MND patients and developed protocols to differentiate these cells into motor neurones. Following characterisation of this cellular model we have identified increased cell death of motor neurones from MND patients in response to protein degradation stress and alterations in proteostatic mechanisms, including ubiquitination, protein aggregation and Ca^{2+} signalling. Our findings yield insight into the link between cellular phenotype and clinical severity.

Conclusion: Both sporadic and familial forms of MND disrupt proteostasis via impairments to protein degradation pathways, resulting in cellular dysfunction, protein aggregation and cell death.

Acknowledgements: This research was funded by the Motor Neurone Disease Research Institute of Australia (MNDRIA). Patient samples were obtained via the MND clinic at the School of Advanced Medicine, Macquarie University, Australia.

SW15 EARLY DYSFUNCTION AND NON-CELL AUTONOMOUS DISEASE MECHANISMS IN A HUMAN IPSC- BASED MODEL OF ALS

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Keywords: iPSCs, motor neurons, electrophysiology

Background: Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disease that remains largely untreatable and incurable, reflecting an incomplete understanding of the key pathogenic mechanisms that underlie motor neuron (MN) loss in the disease. Through the use of induced pluripotent stem cells (iPSCs), we can now study cells from the human central nervous system at a range of time points, including those prior to overt pathology, in order to understand early causative events in ALS.

Objectives: In this study, we report the use of human induced pluripotent stem cell (iPSC)-derived MNs to study the pathophysiology of ALS.

Methods: We have utilised whole-cell patch clamp recording techniques to investigate whether the functional properties of human iPSC-derived MNs are altered in cells derived from ALS patients compared to healthy controls.

Results: We have demonstrated that patient iPSC-derived MNs harbouring C9ORF72 or TARDBP mutations, display an initial hyperexcitability followed by progressive loss of action potential output due to a decrease in voltage-activated Na+ and K+ currents which occurs in the absence of changes in cell viability. Given evidence supporting non-cell autonomous disease mechanisms in ALS, we are currently studying whether interactions between neurons and astrocytes are involved in the pathophysiological phenotype we have recently revealed. Preliminary data suggest that patient iPSC-derived astrocytes can induce pathophysiological changes in controls human iPSC-derived MNs which are similar to those we have recently revealed in patient iPSC-derived MNs. We are currently investigating if such non-cell autonomous mechanisms are common across C9ORF72 and TARDBP lines and whether they rely on direct astrocyte-MN interactions.

Discussion and conclusion: Overall, our data implicate MN dysfunction, potentially due to non-cell autonomous disease mechanisms, as an early contributor to downstream degenerative pathways that ultimately lead to MN loss in ALS.

SW16 INVESTIGATING THE FUNCTIONAL CONSEQUENCES OF RNA PROCESSING DYSREGULATION IN ALS CELL MODELS

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Keywords: inducible cell models, RNA dysregulation, pathophysiology

Background: At least 20 known genetic mutations have been shown to cause ALS. The most common mutations occur in the following genes: *C9ORF72* (uncharacterised function), *SOD1* (superoxide dismutase 1), *TARDBP* (TAR DNA binding protein encoding the TDP-43 protein) and *FUS* (fused in sarcoma). Interestingly, these ALS mutations all result in broad mRNA processing dysregulation. However, it is difficult to interpret whether dysregulation in the expression of any particular gene is a cause or a consequence of the disease. Furthermore, the fraction of aberrantly processed pre-mRNA molecules exported to the cytoplasm and translated into proteins is unknown. This is an important research question since proteins are ultimately involved in cell survival or neurodegeneration.

Objectives: Identify and sequence whole-cell and cytoplasmic mRNA molecules from TDP-43 ALS-inducible cell models in order to investigate at a genome-wide level which aberrantly processed mRNA molecules are exported into the cytoplasm, and which mutations or defects they may carry. Dysregulated factors of interest with known neuroprotective or neurodegenerative functions will be validated at protein level in CNS tissue from ALS patients.

Methods: Build and characterise ALS-inducible cellular models using non-neuronal HEK and murine motor neuron-like NSC-34 cells using the Flp-In system (Invitrogen). Whole cell and cytoplasmic mRNA will be purified before being identified using Next Generation RNA Sequencing (Illumina HiScan). Results are compared to control non-induced cells at various time points following induction of the gene of interest. Quantitative RT-PCR of specific transcripts of interests (altered mRNAs that are unknown or which encode proteins with neuro protective/degenerative functions) and western blotting will be carried out to validate results at RNA and protein levels respectively.

Results: HEK and NSC-34 motor neuron-like TDP-43 cell models of ALS (incorporating either WT or mutant (A315T, M337V, Q331K) forms of human *TARDBP)* have been generated and characterised. Protocols for cytoplasmic fractionation have been optimised to avoid nuclear contamination. Whole cell and cytoplasmic mRNA from induced and non-induced models have been purified and sent for next generation RNA sequencing.

Discussion and conclusion: Generation of stable, inducible, TDP-43 related ALS cell models allows us to investigate RNA dysregulation at early timepoints, as well as during a time course. We anticipate that the characterisations of early dysregulated molecular events in gene expression are likely to be involved in the pathogenesis of TDP-43-related MND. Furthermore, analysis of the cytoplasmic/whole cell mRNA ratio will allow the identification of nuclear-exported mRNA, which is expected to match the proteome. This will distinguish if abnormal cytoplasmic levels of a particular mRNA of interest are due to transcriptional or nuclear export alteration.

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SW17 ESTABLISHING A STABLE CELL MODEL OF C9ORF72-ALS

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Keywords: C9ORF72, RNA toxicity, Cell model

Background: The most common genetic cause of ALS is a (GGGGCC)n hexanucleotide repeat expansion within intron 1 of C9ORF72 (1, 2). It is unknown how the (GGGGCC)n repeat expansion causes ALS pathology, but RNA toxicity is the most attractive hypothesis at present. Therefore, we are investigating the function of (GGGGCC)n repeat RNA using motor neuron cell models.

Objectives: To generate and characterise isogenic motor neuron cell models that express a (GGGGCC)n repeat expansion construct and use these to investigate how the (GGGGCC)n RNA affects cell biology and global gene expression. In addition, develop a reporter assay to screen a small molecule library for therapeutic agents.

Methods: Interrupted (GGGGCC)n repeat constructs of 10, 51 and 102 repeats have been engineered in-house. These (GGGGCC)n constructs have been stably integrated into NSC-34 cells (a murine motor neuron-like cell line) using the Flp-In system (Invitrogen). RNA FISH techniques were used to characterise the cell lines for (GGGGCC)n RNA expression. Further functional assays being developed include ICC, toxicity assays, RNA export assays, splicing assays, microarray and drug screening.

Results: We have successfully generated stable isogenic HEK293 and NSC-34 cells with tetracycline-inducible expression of (GGGGCC)10, (GGGGCC)51, and (GGGGCC)102 RNA, as well as an empty vector control. We have detected nuclear and cytoplasmic RNA foci in the HEK293 and NSC-34 (GGGGCC)n lines, and their size and quantity positively correlate with the (GGGGCC)n repeat length and tetracycline induction.

Discussion and conclusion: We have generated robust cell models C9ORF72-ALS that will be used to study the RNA toxicity hypothesis.

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SW18 STUDYING GLIA-NEURONAL INTERACTION IN C9ORF72 EXPANSION MEDIATED ALS USING AN INDUCED PLURIPOTENT STEM CELL BASED *IN VITRO* MODEL

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Background: Accumulating experimental and human pathological evidence implicates non-cell autonomous mechanisms in the aetiopathogenesis of motor neurone disease. Astrogliosis and glial pathology has long been described in MND but until recently has been assumed to be secondary and / or reactive (1). Recent advances in the genetics of MND have shown that the GGGGCC hexanucleotide repeat expansion on *C9ORF72* is the most common genetic cause of MND (2), human stem cell technologies allows the *in vitro* study of cellular autonomy with a focus on astrocytes.

Methods: Induced pluripotent stem cells (iPSCs) were generated from patients carrying GGGGCC hexanucleotide expansion on *C9ORF72* as well as healthy controls. Following a well-established protocol (3), astrocytes were generated from both control and *C9ORF72* expansion carrying iPSC lines. The function of differentiated astrocytes was assessed by glutamate uptake and calcium imaging. Fluorescent *in situ* hybridisation (FISH) was conducted to detect GGGGCC RNA foci in differentiated astrocytes.

Results: Highly enriched (>90%) functional astrocytes were generated from both control and *C9ORF72* expansion carrying cell lines. GGGGCC RNA foci were detected in mutant astrocytes but not control astrocytes. Evaluation of the influence of genotype on cell viability in isolated and co-culture with neurones under basal and stressor conditions is ongoing.

Discussion and conclusion: Our work has established a platform to investigate the glial pathology and potential non-cell autonomous toxicity of astrocytes in C9ORF72 related ALS.

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SW19 INVESTIGATION OF THE ER AND MITOCHONDRIA CALCIUM CYCLE IN THE PRESENCE AND ABSENCE OF HUMAN G93A MUTATED SOD1

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Keywords: ER, mitochondria, calcium cycle

Background: Neuronal viability depends on regulation of cytosolic calcium. In the pathology of amyotrophic lateral sclerosis (ALS) this is disturbed, eventually leading to motor neuron death (1, 2). Several findings indicate that mitochondrial Ca^{2+} disturbances play a role in ALS (3, 4). Data on ER Ca^{2+} handling, however, are sparse (5).

Objective: The aim of our study is to dissect the individual contribution of mitochondrial/ER calcium uptake and release to ER mitochondria calcium cycle using specific pharmacological tools, further on to investigate their effect on viability of motoneurons in culture in direct comparison to non-motor neurons.

Methods: Motor neurons are prepared from E13 embryonic ventral spinal cord and seeded on astrocytic monolayers. Live fluorescent imaging with cytosolic calcium dyes is performed using FURA-2 at a resolution of 1µm2 / 20Hz, while a rapid perfusion exchange allows <100ms application and removal of drugs. Kainate is used to stimulate calcium permeable AMPA receptors; and caffeine to release Ca^{2+} from caffeine sensitive intracelullar Ca^{2+} stores. The resulting Ca^{2+} transient kinetic of Ca^{2+} influx and intracellular redistribution between organelles like the ER and mitochondria is analyzed.

Results: Preliminary results of this ongoing investigation indicate that in the presence of human G93A mutated

SOD1 mitochondrial calcium uptake and storage is impaired, and endoplasmic reticulum function disturbed with consequent activation of the ER stress response and protein misfolding. Application of caffeine revealed a functional intracellular calcium store in spinal neurons, indicating that ER calcium storage is increased in the hSOD1^{G93A} model of ALS.

Discussion and conclusion: In our current experiments, we are trying to elucidate the connection between overexpression of mutated hSOD1 and ERMCC dysregulation. To answer this, we are collecting further data acquired by blocking or activating ER and mitochondria channels/receptors in charge for fine intracellular $Ca2^{+}$ signalling. Eventually, modulating the ERMCC should allow protective therapy.

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SW19.5 *IN VITRO* ANALYSIS OF GLIAL CELL TOXICITY TO SPINAL CORD MOTOR NEURONS IN THE PRESYMPTOMATIC PHASE OF SOD1G93A ALS MOUSE MODEL - A POSSIBLE INVOLVEMENT OF TNFA, IL-6 AND NGF SIGNALLING

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Background: Amyotrophic Lateral Sclerosis (ALS) is a devastating motor neuron disease that might be due to a combination of cell-autonomous and non-autonomous mechanisms. Microglia and astrocytes seem to be involved in the pathogenesis of ALS, but there is a lack of information on their cell toxicity in presymptomatic phases of the disease.

Objectives: In this study we evaluated the effects of astrocytes and microglia from presymptomatic postnatal day one (P1) and P60 old SOD1^{G93A} (TG) mice and their age matched wild-type (WT).

Methods: Astrocyte or microglia/motor neuron (P1) co-cultures (CC) or treatments with glial conditioned media (CM) were employed in order to analyze motor neuron death (Fluoro-Jade C expression) and axonal retraction (axonal length measurement). The amounts of tumour necrosis factor-alpha (TNFα), interleukin 6 (IL-6) and nerve growth factor (NGF) were quantified.

Results: In general, CC and CM experiments demonstrated that TG microglia (P1 and P60) and TG astrocytes (P1 and P60) led to higher death of WT and TG mouse motor neuron, respectively. The CM of TG microglia (P60) and astrocytes (P1 and P60) altered the final number of WT and TG (microglia) or TG (astrocytes) motor axons. Increased amounts of TNFa, IL-6 and NGF were found in the CM of P60 TG microglia. The levels of above cytokines were altered (elevation of NGF; diminution of TNFα and IL6) in the CM of P60 TG astrocyte. Large gene profiling in ventral regions of spinal cords of P1 and P60 mice revealed the role of biological processes- and signalling pathways-related to cell survival of presymptomatic TG mice. Quantitative polymerase chain reaction showed elevated gene expression levels of the neurotrophic factors CSF1, GDNF, NGF, VEGFA in TG cultured motor neuron compared to WT.

Discussion and conclusion: Cytokines may participate in astrocyte and microglia-induced non-autonomous presymptomatic toxicity to motor neurons. In that stage, autocrine mechanisms *in vivo* may rescue motor neurons from degeneration.

Acknowledgements: This study was supported by FAPESP and CNPq

SW20 EXTRACELLULAR AGGREGATED SOD1 IS TOXIC TO ASTROCYTES

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Keywords: SOD1, aggregated, astrocytes

A large body of literature suggests that amyotrophic lateral sclerosis pathology is initially linked with neuroinflammation specifically gliosis. However the actual cause of gliosis remains unclear. Mutations in the superoxide dismutase 1 (SOD1) gene account for 20% of familial cases of ALS and misfolded SOD1 has been associated with sporadic ALS. As protein aggregates are a hallmark of all ALS cases we sought to determine the effect of aggregated SOD1 on astrocytes. When added to astrocytes, aggregated SOD1 was internalized rapidly and remained within primary astrocytes for up to 96 hours. Aggregated SOD1 when internalized by primary astrocytes has a toxic effect and escapes into the cytosol. This work provides a potential link between gliosis and protein aggregation in ALS.

SW21 HSOD AND THE ALS ASSOCIATED MUTATION G93A INDUCE SPECIFIC LIPIDOMIC PROFILES IN CENTRAL NERVOUS SYSTEM IN TRANSGENIC MICE

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Keywords: biomarkers, lipidomics, central nervous system

Lipids are essential for neuronal function. Previous work from our group revealed significant changes in fatty acid profiles, in both spinal cord and brain cortex from ALS patients, in comparison with age and gender-matched individuals (1). To offer a comprehensive view of the potential changes associated with motor neuron demise in ALS, we studied the lipidomic signatures of spinal cord and brain cortex from transgenic mice $hSOD^{G93A}$ (high copy number), in comparison with hSOD wt and non-transgenic littermates.

We analyzed the influence of age on these parameters by measuring samples from early preclinical phase (30 days) to end-stage disease (120 days). The results show that: i) in both spinal cord and brain, age is a stronger factor than transgenesis in lipidomic profile determination; ii) despite being phenotypically identical, hSOD wt transgenic mice exhibit a differential lipidomic profile comparing to non-transgenic littermates in both early and end-stage of the disease, suggesting that transgenesis is able to impinge reactive changes on the lipidome; iii) accordingly, in spinal cord, transgene (independently of the mutation) has a high impact in lipidome profile; iv) the number of differential molecules between hSOD wt and $hSOD^{G93A}$ spinal cord mice increase with mice age; and v) at 30 days, $hSOD^{G93A}$ transgene induce changes in lipids belonging to phospholipids, sphingolipids and glycerolipids. Globally, these data illustrate the importance of lipidomic changes in this paradigm of neurodegeneration, suggesting the importance of lipid-related novel pathogenic pathways associated with motor neuron dysfunction.

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SW22 STEM CELL SURVIVAL IN THE SOD1 RAT MODEL OF ALS

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Keywords: stem cell therapy, Transplantation, SOD1 G93A

Background: Stem cells can be used to create motor neurons under tightly controlled laboratory conditions. These cells may be implanted into the brain and spinal cord of rodent models of MND. We have found that these transplanted cells delay the development of motor symptoms, although this delay is only transient. We have also noted that transplanted cells do not survive for long enough to provide a more lasting benefit. We speculated that either the host immune system *or* the MND pathology itself is influencing the survival of these cells.

Objectives: 1) To identify critical features of graft rejection in wild-type rats; 2) to improve the survival of cells implanted into the SOD1 rat.

Methods: Twenty-four WT neonate rats and 12 athymic adult rats received intra-striatal injections of 1.5 × 105 cortical progenitor cells, derived from a GFP expressing human embryonic stem cell line. Animals were divided into 4 time-points (WT n=6/group; athymic n=3/group): 4 weeks, 6 weeks, 8 weeks and 12 weeks. Animals were euthanized at the given time-points and analysis of graft volume and survival performed.

Eleven SOD1 rats received a graft of cells as detailed above. Eight of these animals received daily subcutaneous injections of cyclosporin A (10mg/kg). All animals were assessed for motor performance on the rotarod from 12 weeks of age.

Results: While graft volume reduced as a function of time in WT animals, this reduction did not reach significance ($p = 0.214$). However, graft volume *increased* over the same period in athymic animals ($p = 0.0064$). Animals treated with cyclosporin A died earlier (133 ± 22 days) compared to untreated animals (142 ± 11 days). Treated animals also performed worse on the rotarod from 20 weeks ($p = 0.037$).

Discussion and conclusions: The main finding from the research project was that the host-immune system, rather than the MND-like environment is having the major impact on graft rejection. This is a positive and important result because it suggests there is scope for further improving the efficacy of stem cell grafts by promoting long-term survival through suppression of the host immune system. A second important and unexpected finding was that suppression of certain aspects of the host immune-system (in order allow for better survival of the grafted cells) actually made the disease progression worse. In summary, these results show that while there is good support for the general principal that stem cells can have therapeutic benefit for MND, there are important but surmountable practical hurdles related to the host immune-system that must be addressed if we are to continue basic research that can lead to development of effective stem cell therapies with long-term benefits to the patient.

Acknowledgments: Florey Institute of Neuroscience and Mental Health; Motor Neurone Disease Research Institute of Australia; University of Melbourne

SW23 CELLULAR THERAPY OF ANIMAL MODEL OF AMYOTROPHIC LATERAL SCLEROSIS BY TRANSPLANTATION OF HUMAN ASTROCYTES DERIVED FROM PLURIPOTENT STEM CELLS

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Keywords: hPSC, astrocytes, SOD 1 mice

Amyotrophic lateral sclerosis (ALS) is characterized by death of motor neurons (MNs) in the CNS. Yet, cellular abnormalities are not limited only to motor neurons. Many studies suggested that malfunctioning astrocytes in both ALS patients and in rodent ALS models underlie the disease.

Kadimastem has developed a unique and robust protocol for generating a highly homogenous population of astrocytes (>90% GFAP, S100b) from human pluripotent stem cells (hPSC). Our differentiation protocol, together with our scalable culturing technology, allows us to produce large quantities of astrocytes *in vitro*. These hPSC-derived astrocytes exhibit similar gene expression patterns to primary human astrocytes as well as functional properties that include: i) Secretion of neurotrophic factors that protect motor neurons (BDNF, GDNF and VEGF); ii) Capacity of glutamate uptake; iii) Protection of neurons from oxidative stress *in vitro*. These hPSC derived astrocytes can be kept frozen and used upon need.

We assessed the therapeutic properties of these hPSC-derived astrocytes *in vivo*. For that aim the cells were transplanted intrathecally into high-copy number $hSOD1^{G93A}$ mice (a mouse model for a severe form of ALS). Human astrocytes transplantation resulted in significant improvement ($P<0.05$) in motor performance in all functional tests. In addition, positive effects on survival and delay in onset of disease were observed in transplanted mice. The results show that hPSC-derived astrocytes could modify the course of ALS.

SW24 NEW MOLECULAR THERAPY FOR AMYOTROPHIC LATERAL SCLEROSIS WITH SPINAL CORD TARGETING PEPTIDES

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Keywords: siRNA, targeting, molecular therapy

Background: A number of treatments have been tested in ALS animal models and patients, but no satisfied treatment has been established yet. Here, we performed a new therapeutic strategy to deliver siRNA-SOD1 combined with spinal cord homing peptides.

Objectives: The engineering of a new molecular therapy for ALS by siRNA technology with specific targeting peptide to spinal cord.

Methods: We screened candidates of spinal cord targeting peptides by using *in vivo* phage display methods. We injected 10^{12} titre of phage from randomized phage library into C57BL6 mice through the tail vein. We repeated the bio panning five times. After that, we selected two major candidates (SP1 and SP2) as spinal cord specific targeting peptides.

We evaluated the affinity of the candidate peptides (SP1 and SP2 peptides) with spinal cord. We injected 10^{12} titre of each phage, which expressed SP1, SP2 or randomized peptides (phage library). After injection, we collected and counted the number of the binding phage with spinal cord, brain or non-nervous organs (heart, kidney and liver). At the same time, the sections of spinal cord were cut and immunostained with anti-phage antibodies.

We analyzed the stability and binding potential between the siRNA-SOD1 oligonucleotides and SP1 or SP2. We prepared the mixture series of weight ratio between siRNA and peptides, which were electrophoresed to agarose gels to determine the best weight ratio between two.

Finally, we treated human $SOD1^{G93A}$ transgenic mice (ALS model mice) with siRNA-SOD1+SP1 or siRNA-SOD1+SP2 injection once or three times per week through tail vein. The effects were evaluated by Rota-rod test and western blots and immunohistochemistry of human SOD1 expression.

Results: We identified two candidate peptides (SP1 and SP2) of high affinity with spinal cords. The affinities were much higher than in not only brain, but also heart, kidney and liver. Phages expressing SP1 or SP2 accumulated in spinal cord compared to phage library. The peptides could bind with siRNA oligonucleotides by the electrostatic uniter. The best ratio of the weight between the peptides and the oligonucleotides was 3:1. siRNA-SOD1+SP1 or siRNA-SOD1+SP2 delayed the progression of motor function loss compared to non treatment disease mice. $hSOD1^{G93A}$ expression was inhibited with siRNA-SOD1+SP1 or siRNA-SOD1+SP2 in spinal cord.

Conclusion: We identified two spinal cord targeting peptides for the delivery of small oligonucleotides to specific tissues, which has high potential to provide a new molecular therapy.

SW25 CLINICAL TRIAL OF CEFTRIAXONE IN SUBJECTS WITH ALS - POST-HOC ANALYSIS USING CEFTRIAXONE CUMULATIVE DOSE

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Keywords: Ceftriaxone, clinical trial, cumulative dose

Background: Ceftriaxone, a semi-synthetic, third generation cephalosporin antibiotic, has been demonstrated to increase the level of a glutamate transporter that clears glutamate from the synapse. Studies of ceftriaxone in the laboratory suggest that it may protect motor neurons from injury.

Participants were randomly assigned to receive treatment with ceftriaxone (2/3 of participants) or placebo (1/3 of participants) for at least 12 months. Statistical analyses evaluated survival and functional decline based on the revised ALS functional rating scale (ALSFRS-R). No significant effect was found.

The reasons of failure to detect efficacy could include medication adherence and treatment interruption due to side effects. Though intention-to-treat analysis was used in the primary analysis, we are planning a post-hoc analysis to evaluate the extent to which ceftriaxone may have been effective based on the cumulative dose of the drug, to account for deviations from the planned dosing regimen. This post-hoc analysis was approved by the study steering committee.

Objectives: In the clinical trial of Ceftriaxone in Subjects with Amyotrophic Lateral Sclerosis (ALS), approximately 500 subjects were randomized at 70 centers in US and Canada.

The trial did not demonstrate significant efficacy in ceftriaxone arm compared to placebo arm by intention to treat analyses, however treatment adherence may have masked the efficacy of ceftriaxone. In this post-hoc analysis, we will examine the effects of cumulative dose of ceftriaxone, and estimate the plausible effect of the drug on the disease process, to see if there was adequate power to detect the efficacy.

We will assess whether 4g/day ceftriaxone infusion has any significant positive effect to 1) survival; 2) the rate of decline in function compared with placebo arm.

Methods: The co-primary outcome measures for this post-hoc analysis will be the same as those in the primary trial: difference in 1) survival and the 2) rate of decline in function as measured by the revised ALS functional rating scale (ALSFRS-R) between the treatment and placebo groups at the end of study. We will also analyze secondary outcome measures including vital capacity, and evaluation of multiple upper and lower extremity muscles using hand held dynamometry.

Cumulative effect of ceftriaxone is assessed by summation of calculated daily changes of severity compared with those of placebo arm. Values will be adopted only when the patients are taking ceftriaxone in the active group.

SW26 RILUZOLE AS A RISK FACTOR FOR VTE IN ALS

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Keywords: riluzole, venous thromboembolism, ALS

The risk of VTE is increased in many Neurological and Neurosurgical patients such as stroke and spinal cord injury; however, weakness, alone is not the only predisposing factor to such events or else almost all Neurological diagnoses would have this comorbidity. As an example, a recent study of MS patients actually didn't indicate an increased risk of VTE. Although a paucity of data exists, the trend does demonstrate an increased risk of VTE in ALS patients. Past studies have demonstrated an association with lower functional status, ie restricted mobility and lower respiratory function, and elderly age to have an increased risk of VTE in ALS patients. To the best of our knowledge, however, there have not been any other factors shown to contribute to the increased risk of VTE in patients with ALS. It is important to understand potential risk factors contributing to the increased incidence of VTE in ALS patients; as a result, the current study investigated the risk of riluzole, the only medication approved and commonly prescribed for ALS, in VTE. A retrospective chart review of patients with the diagnosis of ALS at our institution was performed over 8 years to determine the incidence of VTE and to identify potential risk factors, specifically, riluzole. Lower extremity weakness was associated with an increased risk of VTE. Riluzole was not associated with an increased risk of VTE.

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SW27 THE DEVELOPMENT OF MESSAGE BANKING AS A PATIENT-DRIVEN CLINICAL TOOL

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Keywords: dysarthria, message banking, communication

Background: 'Message banking' is a relatively unexplored Speech and Language Therapy intervention for progressive communication disabilities associated with MND. This involves facilitating patients to record themselves saying short messages prior to deterioration of speech. These are typically everyday messages and often phrases unique to that person. These recordings can be used at a future stage for the speech output of pre-stored messages on an electronic communication device (ECD). Such an intervention provides not only a means of maintaining interaction, but may also be a powerful way for individuals to navigate their changing identities as communicators.

Objectives: To explore the experiences of three patients engaged in a message banking process in order to inform future implementation of this intervention and to guide a larger proposed clinical study.

Methods: A focus group was conducted with 3 individuals with progressive communication disabilities (two people with MND and one with primary progressive aphasia) who were all engaged with message banking. The data was transcribed and analysed qualitatively using Thematic Analysis.

Results: Emergent themes suggested: a desire for information and rapid delivery of that information after MND diagnosis; the notion of choice and future options as empowering; the value placed on the messages banked; and uncertainty. Practically, there were differing opinions on the best process for recording messages, and a common frustration with regards to reported delays in implementation on an ECD when other services were engaged.

Discussion and conclusions: This small dataset on an innovative and emerging area of practice suggests that message banking may play an important role in facilitating people with MND to negotiate the changing landscape of their communication abilities. Practical and clinically sound solutions need to be found to inform SLT practice with regards to supporting patients in strategically identifying relevant messages and designing, programming and implementing patient-specific communication content within ECDs. How to address these questions will be discussed in our proposed follow-up study.

THEME 12B CLINICAL WORK IN PROGRESS AND CARE PRACTICE

CW1 TO DO OR NOT TO DO? ADVISING LEVEL OF 'DOING' IN MND

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Keywords: rehabilitation, occupation, exercise

Background: At the International Symposium for ALS/MND 2013 the author presented a poster titled "Purposeful Occupation is Effective in Treating Motor Neurone Disease" which challenged the concept of adaptation that occupational therapists presently adopt in interventions with MND. The poster promoted great discussion. This question has now gained PhD Fellowship Funding and the author is subsequently in the second year of this study.

Objectives: To develop rehabilitative intervention which focuses on enabling engagement in occupation; To explore patients experiences of rehabilitative approach and to generate evidence of rehabilitative approach to inform further research

Method: An exploratory study for feasibility of delivering alternative rehabilitative Occupational Therapy intervention.

A convenience sample of 12 people living in North Wales diagnosed with MND was used in this study. People with MND are entitled to receive statutory occupational therapy provision, termed as the 'usual' treatment. A comparison group, who receive this 'usual' treatment only, are utilized.

6 people, randomly selected, received the 6-week rehabilitative intervention which consists of weekly occupational therapy sessions in their own home incorporating functional activity which empowers them to work to moderate aerobic capacity and moderate muscle resistance.

Both groups are evaluated via Canadian Occupational Performance Measure (COPM) (2) which provides quantitative data in terms of the performance and satisfaction of performance over time. Qualitative data is obtained through semi-structured interviews with participants at 3-time points.

An inductive approach to data analysis is utilised for the qualitative data and the interpretive strategy will be based on adaptation of the constant comparative method. The quantitative date will be analysed through repeated measures anova.

Discussion and conclusion: There is developing evidence that regular aerobic and resistance exercise can maintain respiratory function in mild to moderate presentations of MND (1). Combine this with the knowledge that engagement in purposeful occupation is being consistently proven to improve health and well being in a variety of chronic conditions (3).

Then combine this with international MND specialist therapists experience that those people with MND who actively 'push themselves' to engage in activity appear to achieve a plateau in function whilst those with occupational deprivation have a more marked decline.

The presentation will focus on presenting an argument for a cautious change in practice for therapists working with people with MND/ALS.

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CW2 A CASE OF MOTOR NEURONE DISEASE OF THE BROWN-VIALETTO-VAN LAERE TYPE WITH ONSET IN THE FOURTH DECADE

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Keywords: Brown-Vialetto-Van Laere syndrome, pontobulbar palsy, riboflavin

Background: Brown-Vialetto-Van Laere (BVVL) syndrome is a rare mostly paediatric form of motor neurone disease manifesting as pontobulbar palsy with sensorineural hearing loss.

Objective: To describe a case of BVVL syndrome with onset in the late fourth decade.

Methods: Clinical history and examination, neuroimaging, clinical neurophysiology studies, and laboratory investigations.

Case Report: A 39-year-old Caucasian woman came to our Institute with a 14-month history of progressive dysarthria, nasal voice and dysphagia. She also complained of bilateral tinnitus; no limb weakness was reported.

The patient had no major medical antecedents. Family history was notable for presenile hypoacusis in paternal granfather, father, and two paternal uncles, one of whom was also demented. On neurologic examination, she was

severely dysarthric and rhinolalic. Bilateral facial weakness was evident, as well as tongue weakness with atrophy and fasciculations; elevation of the soft palate was limited. The limbs showed generalized hyperreflexia but normal muscle mass and strength and no fasciculations; cutaneous plantar response was flexor bilaterally. The remainder of the examination was normal.

Brain MRI showed modest platybasia with normal brainstem signal. Electromyography disclosed marked chronic neurogenic changes associated with severe active denervation in the genioglossus muscle bilaterally, whereas no signs of denervation were detected in limb muscles and nerve conduction studies were normal. Routine blood tests were notable for elevated cholesterol (261 mg/dL) and a low vitamin B12 (85 ng/L, normal range 211-911) without anti-gastric parietal cell antibodies; cerebrospinal fluid was normal. Audiometry and brainstem auditory evoked potentials demonstrated bilateral sensorineural hearing loss.

On the basis of the phenotype of pontobulbar palsy with demonstration of sensorineural hearing deficit, diagnosis of motor neurone disease of the BVVL type was made.

Discussion and conclusions: BVVL syndrome is a rare disease presenting mostly in pediatric age of which only 74 cases had been reported until 2012 (1). Recently it has been demonstrated that a considerable proportion of patients carry recessive mutations in the riboflavin transporter genes SLC52A3 and SLC52A2, and some of them improve following riboflavin supplementation, making this syndrome a prominent example or treatable motor neurone disease (2). Based on such evidence, we have prescribed a course of oral riboflavin supplementation and we are waiting to re-evaluate the patient. We are also planning to perform genetic studies of riboflavin transporters, possibly evaluating other family members in search of compatible disease features.

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CW3 NUMBER OF C9OR72 REPEAT EXPANSIONS AND PHENOTYPIC CORRELATION IN ALS

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Introduction: It is known that hexanucleotide expansion (GGGGCC) in the C9ORF72 gene is associated with ALS pathogenesis but the range of normality established for the number repeats of this expansion is controversial in the scientific literature. Nevertheless, currently 20-22 repeats is the minimal range associated with FTD and 30 repeats with ALS.

Objectives: Our objective was to analyse the clinical phenotype of three groups of patients defined by the number of hexanucleotide repeat expansion as follows: 2, 8-19 and over 30 repeats.

Methods: We performed a retrospective analysis of 81 patients. We defined three groups according to the number of repeats: 7 patients with 2 repeats (normal); 30 with 8 to 19 repeat expansions (intermediate); and 5 with more than 30 (pathological). Variables analyzed were: age of symptom onset; sex; family history of neurodegenerative disease; type of onset; cognitive impairment; time of survival at the last visit.

Results: In the subgroup of 2 repeats expansions: 5 were men; the average age of onset was 58 years (range 32-72 years); 4 had bulbar onset and 3 spinal; 4 out of 6 patients had cognitive involvement; 20% had familial history of neurodegenerative diseases; the average survival time was 27 months (range 8-54 months).

In the subgroup with 8 to 19 repeat expansions: 67% were men; the average age at diagnosis was 61 years (range 35-79 years); 87 % had spinal onset and 13% bulbar; 3 out of 13 patients had cognitive impairment;17% had family history; the average survival time was 21 months (range 4-71 months).

In the subgroup of more than 30 repeat expansions; 3 were men; the average age of onset was 61 years (range 48-77 years); 3 had spinal onset and 2 bulbar; 3 had cognitive impairment; only one patient had family history of neurodegenerative diseases (ALS); the average survival time was 21 months (range 12-30 months).

The statistical analysis did not show any significant difference between any subgroup ($p>0.05$).

Discussion and conclusion: ALS patients with over 30 repeats in C9ORF72 showed no difference in terms of age, sex or family history when compared with those of intermediate or normal number of repeats. The presence of cognitive impairment in our population doesn't predict positivity for C9ORF72 mutations. In patients with C9ORF72 mutations, having >30 had a non-significant trend to more cognitive impairment. Cognitive impairment was not related to shorter survival time.

CW4 MONOCYTE SUBTYPES IN ALS

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Keywords: monocyte, neuroinflammation, immunmodulation

Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that is characterized by progressive loss of motoneurons (1). Neuronal loss in ALS is accompanied by a neuroinflammatory reaction including recruitment of peripheral monocytes to the CNS (2). Peripheral human monocytes are dividable into two subpopulations, the pro-inflammatory $CD14^{++}CD16^{-}$ monocytes in and the regenerative $CD14^{+}CD16^{++}$ monocyte subtype (3).

Objectives: The aim of this study is to decipher the contribution of the different monocyte subpopulations to disease initiation and progression in ALS patients, preclinical ALS mutation carriers and in an ALS mouse model.

Methods: The methods used are flow cytometry, isolation and culture of primary human monocytes, ELISA and immunohistochemstry.

Results: To analyze the composition of monocyte subtypes we have measured the monocytes subpopulations in the blood of ALS patients, preclinical carriers of ALS mutations and healthy, age-matched controls by flow cytometry. We found that the equilibrium of monocyte subtypes is shifted to the proinflammatory side in ALS, preclinical carriers of ALS mutations as well as in $SOD1^{G93A}$ Tg mice. In ALS mice, the monoycte subtype composition is dependent on the host organ and the stage of disease. For further analysis of the properties of human monocytes *in vitro* we developed an isolation and culture system for the pro-inflammatory $CD14^{++}$ monocyte subtype. Currently, experiments concerning $CD14^{++}$ monocyte activity and cytokine secretion, adherence properties and chemotactic behaviour upon LPS stimulation and immunmodulating agents are ongoing.

Discussion and conclusions: Peripheral monocytes infiltrate the CNS and contribute to motoneuronal loss in ALS mice (2). We found, that the equilibrium of monocyte subtypes in ALS patients, preclinical mutation carriers and ALS mice is shifted to the proinflammatory side *in vivo*. This finding indicates an involvement of especially the CD14++ monocyte subtype to ALS progression, thereby presenting a possible target for therapeutic intervention.

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CW5 PROTEOMIC ANALYSIS OF MUSCLE TISSUE FROM PATIENTS WITH MOTORNEURON DISEASE AND CONTROLS

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Keywords: mass spec, proteomics, biomarker

Objectives: Is there a difference in muscle proteome in patients with ALS compared to controls when analysed with mass spectroscopy (MS) and can that difference be used as a biomarker?

Methods: A muscle biopsy was taken from the anterior tibial muscle in 10 ALS-patients and 10 controls without neurological disease. Three controls with other neurological diseases with denervation were also analysed in order get a picture of proteomic change due to denervation. Muscle biopsies were homogenized and the proteins were extracted with detergent lysis buffer. Extracted proteins were on-filter digested with trypsin. Dimethyl isotope labels were used to globally label the tryptic peptides for relative quantification. Individually labelled peptides from patients and controls were combined and analysed by reversed phase nanoliquid chromatography and mass spectroscopy. The same procedure was repeated but with different labels for patients, controls and denervation controls.

Results: Eleven proteins were found in significantly different abundance in patients compared to healthy controls. Of those, two proteins were the most interesting, Cytochcrome C oxidase and Valocin containing protein (VCP).

Analyses including controls with denervation due to other neurological diseases did not show the same abundancy changes, suggesting that the differences found between ALS-patients and healthy controls were not only due to denervation.

Conclusions: Muscle proteome analysed with MS is different in patients with ALS as compared to controls. This difference does not seem to be due to denervation solely. Proteomic analysis of muscle tissue might be used as a biomarker for the disease.

CW5.5 CHOLESTEROL METABOLITES REGULATE MOTOR NEURON SURVIVAL VIA LIVER X RECEPTORS

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Keywords: cholestenoic acid, cholesterol, mass spectrometry

Background: Cholestenoic acids are metabolites of cholesterol which are synthesised in the CNS. Here we profiled the cholestenoic acid content of human cerebrospinal fluid and identified two acids as ligands to the liver X receptors (LXRs).

Results: We found that one of these acid, 3β, 7α-diHCA (3β,7α-dihydroxycholestoic acid), promoted oculomotor neuron survival in an LXR-dependent manner in mouse *in vitro* and *in vivo*. Another acid, 3β-HCA (3β-hydroxycholestenoic acid), caused motor neuron cell loss in mice.

Two neurological diseases, hereditary spastic paresis type 5 (SPG5) and cerebrotendinous xanthomatosis (CTX) which may present with spastic paresis both show disturbed cholestenoic acid biosynthesis. In both diseases the level of 3β, 7α-diHCA is reduced, while in CTX the level of 3β-HCA is also elevated. Our results indicate that specific cholestenoic acids selectively work on motor neurons to regulate the balance between survival and death.

Discussion and conclusion: Our data suggest that 3β, 7α-diHCA may be a pharmaceutical lead compound for treatment of motor neuron disorders, and that the cholestenoic acid profile of CSF could be a biomarker for motor neuron disease.

CW6 DEVELOPMENT OF A NEW PROTEIN BIOMARKER PANEL USING SELECTION REACTION MONITORING MASS SPECTROMETRY (SRM-MS)

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Keywords: biomarkers, SRM, proteomics

Background: There is a strong need for the identification of biological markers for the prognostic stratification of amyotrophic lateral sclerosis (ALS), an invariably fatal motor cell disorder. Any meaningful biological indicator to be used for diagnosis or for monitoring purposes is likely to be represented by a panel of different molecular signals (1). The present study has been designed to track the expression of proteins that could be associated with the pathogenesis of this disease in different biofluids. Isobaric tandem mass tag labelling in combination with selected reaction monitoring (TMT-SRM) has emerged as a promising high-throughput targeted protein quantification technology for biomarker verification. In this study TMT-SRM has been successfully applied on CSF samples of ALS patients and control for the analysis of proteins that have been previously proposed as ALS-related biomarkers (2-4). 20 protein candidates have been preliminary selected and measured simultaneously to corroborate their potential as a panel of biomarkers in ALS.

Objectives: Our objective is to develop a panel of protein biomarkers that can be used to discriminate between ALS and other Neurodegenerative diseases and to distinguish between different phenotypes of the disease

Methods: A pool of 8 CSF samples (4 controls and 4 ALS patients) was used for the method optimization. The SRM assay was optimized using a TSQ Vantage mass spectrometer that generated highly accurate peptide quantification. A second universal CSF pool was used for the reproducibility test of human recombinant proteins in order to improve recoverability.

Results: Six proteins could be unequivocally detected and measured using the TMT-SRM method, showing an interesting perspective of high-throughput and sensitive measurements of disease-relevant proteins by using this proteomic approach. Further investigations will be performed to detect the remaining proteins in our 20-target panel and increase the potential of our biomarkers investigations into ALS.

Discussion and conclusions: TMT-SRM is a highly specific and sensitive technique for protein detection, providing reproducible measurement of proteins in a biological fluid. Additionally, further optimization of this technique is likely to allow the extensive analyses of other disease-relevant proteins. This is a preliminarily important step towards the establishment of an assay for a panel of proteins which overcomes the limits imposed by the use of antibody-based ELISA assays and contributes to the establishment of biomarkers for the recognition and monitoring of ALS.

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CW7 EVALUATION OF CORTICOSPINAL TRACT ALTERATION WITH DOUBLE INVERSION RECOVERY MAGNETIC RESONANCE IMAGING AS A NEW DIAGNOSTIC MARKER IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: magnetic resonance imaging, diagnosis, pyramidal tracts

Background: In amyotrophic lateral sclerosis (ALS), MRI provided corticospinal tract (CST) degeneration and may have a diagnostic contribution. However, the diagnostic value of conventional T2 and FLAIR MRI is not high. This study was performed to prospectively determine the sensitivity in the detection of CST lesions by using double inversion recovery (DIR) MRI in patients with ALS.

Methods: Seven patients presenting with a clinically definite or probable of ALS of revised El Escorial criteria, and 7 age and sex matched control subjects were included in this study. Imaging was performed on a 3T MRI using DIR and FLAIR sequences. Region of Interest (ROI) of the CST were classified according to 6 regions: precentral gyrus; corona radiate; centrum semiovlae; interal capsule; crus cerebri; basis pontis. Quantitative analysis (ie contrast-to-noise ratio and relative contrast) and visual scale were analyzed between ALS and control groups. MR images were analysed by two of the investigators, independently.

Results: The visual analysis of 6 ROIs did not have significant difference between two groups in all sequences. However, the quantitative evaluation of DIR revealed a significant increase of the signal intensity at the almost CST ROIs in ALS patients. On the other hand, there was no significant signal change between ALS and control groups in quantitative measurement of FLAIR sequence.

Discussion and conclusion: DIR brain MRI at 3T provides the high sensitivity compared to conventional MRI in the diagnosis of ALS and may be useful for diagnosing ALS.

CW7.5 ANATOMICAL CONNECTIVITY MAPPING AS A COMPLEMENTARY MEASURE TO FA IN ALS

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Keywords: anatomical connectivity mapping, FA, VMB

Background: Diffusion weighted imaging (DWI) is useful in characterising white matter changes in patients with ALS. Previous studies have focused on fractional anisotropy (FA) demonstrating abnormal values particularly in the corticospinal tract and in the corpus callosum of patients with ALS compared to healthy controls. These changes are usually interpreted as an index of degeneration along specific tracts. Recently, a DWI measure of anatomical connectivity, namely anatomical connectivity mapping (ACM) was introduced, and applied to Alzheimer's disease and Multiple Sclerosis demonstrating its sensitivity to pathological processes different from those typically highlighted by FA changes.

Objective: The aim of the study was to apply ACM to DWI data of patients with ALS to assess the potential of this parameter for the study of in ALS. In particular, we were interested in assessing the complementarity of FA and ACM in characterising the pathology on ALS *in vivo*, and in detecting patterns of altered connectivity.

Methods: This is a retrospective study based on data from 24 subjects with ALS (mean age 52.7 ± 12.82) and 24 healthy controls (mean age 47.2 ± 9.37). DWI data were acquired on a 3T MRI scanner (TE=104.5ms; maximum b factor=1300smm^{-2}; number of diffusion encoding directions=32).

The images were corrected for involuntary motion and eddy current distortions using affine registration and the FMRIB's Linear Registration Tool (FLIRT). The single tensor (ST) model was used to derive mean diffusivity (MD) and FA, while the persistent angular structure (PAS) MRI model was used to resolve up to 3 directions per voxel and then to compute ACM. All the fitting and tractography were performed using Camino.

All the images were normalised into MNI space using ANTs 1.9.x, and smoothed with 6 mm^3 full-width at half-maximum Gaussian filter.

Statistical analysis was carried out using SPM8, to compare FA, MD and ACM between groups.

Results: The FA results, at an uncorrected level of $p<0.005$, demonstrate reduced FA in ALS subjects compared with controls, extending continuously from the subcortical CST fibres to the brain stem. These results survive FWE correction at cluster level ($p<0.05$).

The ACM results, at an uncorrected level of $p<0.005$, demonstrated increased connectivity in ALS subjects compared to controls, primarily located in two large bilateral clusters in the white matter of the corona radiata in close proximity to the superior and middle frontal gyrus. There were no significant differences shown in MD between healthy controls and ALS subjects.

Discussion and conclusion: ACM shows more extensive CST damage than has been apparent in many DTI studies in ALS. Our results do not support any simplistic views of "dying back" versus "dying forwards" mechanisms of CST degeneration in ALS.

Our findings of increased connectivity may reflect compensatory mechanisms, functional and/or structural.

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CW8 HIGH AND ULTRA-HIGH FIELD MR SPECTROSCOPY IN ALS

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Keywords: magnetic resonance spectroscopy, biomarkers, imaging

Background: Magnetic resonance spectroscopy (MRS) is promising as a biomarker in ALS because it allows noninvasive investigation of neurochemical changes in the brain, which has potential value both as a marker of disease severity and as an opportunity to study the pathophysiology of ALS *in vivo*. Glutathione (GSH) deficits are a marker for oxidative stress, which has been implicated in motor neuron degeneration in ALS (1). Decreased motor cortex GSH levels in ALS were recently reported at 3T using edited MRS (2). However, decreased motor cortex GSH levels remain to be confirmed with short echo MRS that will not be confounded by T2 changes. Another recent abstract reported reductions in the neuronal marker NAA and the neurotransmitter glutamate in the motor cortex in ALS (3).

Objectives: In order to extend the preliminary observations noted above, we are using edited MRS to obtain GSH levels at 3T and short-echo, non-edited MRS to obtain a neurochemical profile including GSH at 7T. This protocol is expected to distinguish between GSH concentration changes and changes in the relaxation properties of the tissue and conclusively determine if there is a GSH deficit in the motor cortex in ALS.

Methods: Subjects with El Escorial possible, probable, and definite ALS, as well as age-matched control subjects were studied. ALS subjects are characterized by site of onset, neurologic examination, ALSFRS-R, and Edinburgh Cognitive Assessment Scale (ECAS) (4). Non-edited MRS data at 7T are obtained using a protocol that was previously demonstrated to provide a neurochemical profile consisting of about 15 metabolites (5). Edited MRS data at 3T are obtained using previously described methods that allow reliable GSH quantification at 3T and 4T (6, 7).

Results: We will present preliminary data on MRS findings of GSH and other metabolites in ALS subjects as compared to non-affected controls, as well as on potential correlations between clinical and MRS measures.

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CW9 EFFECTS OF THE ENDOCANNABINOID SYSTEM MODULATION ON EXCITOTOXIC STRESS OF RAT HYPOGLOSSAL MOTONEURONS

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Keywords: hypoglossal motoneurons, excitotoxicity, endocannabinoid

Background: Brainstem hypoglossal motoneurons (HMs) exclusively innervate tongue muscles and are the first ones to be damaged in the bulbar form of amyotrophic lateral sclerosis. One mechanism leading to such cell death is proposed to be glutamate-mediated excitotoxic stress. HMs are particularly vulnerable to excitotoxicity due to their expression of calcium permeable AMPA receptors (1) and scarcity of

intracellular Ca^{2+} binding proteins like parvalbumin and calbidin (2). Indeed, blocking glutamate uptake in medullary slices can lead to pathological bursting and motoneuron damage (3). The endocannabinoid system is widely distributed in the brain and is believed to be important as a regulator of synaptic transmission. In ALS animal models up-regulation of the endocannabinoid system has been detected, suggesting it can play a role during disease development (4).

Objectives: Our objective was to investigate the effect of modulation of the endocannabinoid system on the excitotoxic stress in HMs of the rat.

Methods: We used thin medullary slices from postnatal Wistar rats. Each slice, containing hypoglossal nuclei, was transferred to a recording chamber and superfused with oxygenated Krebs solution. Whole cell patch-clamp recordings were performed on HMs, while drugs were administered via the perfusion system. Excitotoxic stress was evoked by application of DL-TBOA (DL-threo-β-benzyloxyaspartic acid, 50μM), a potent and selective inhibitor of excitatory amino acid transporters with consequent buildup of extracellular glutamate.

Results: Activation of the cannabinoid receptor 1 (CBR1) in HMs is reported to decrease glycinergic transmission (5). In our study, the CBR1 agonist anandamide (AEA, 10μM) significantly decreased also spontaneous excitatory post-synaptic currents. Furthermore, modulation of CBR1 function affected TBOA-evoked bursting. In fact, co-application of AEA with TBOA resulted in lowered probability of the occurrence of pathological bursting, whereas co-application of the CBR1 antagonist AM251 (10μM) disrupted TBOA induced bursts, leading to their fragmentation in the majority of recorded cells.

Discussion and conclusions: Our data suggest that the endocannabinoid system plays an important role in the HM hyperexcitability due to excitotoxic stress. Further studies are necessary to determine if up-regulation of CBR1 can help cell survival during excitotoxic injury.

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CW10 THRESHOLD TRACKING REVEALS CHANGES OF PERIPHERAL AXONAL EXCITABILITY IN HEREDITARY SPASTIC PARAPLEGIA

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Keywords: hereditary spastic paraplegias, threshold tracking, axonal excitability

Background: Hereditary spastic paraplegias (HSP) are highly heterogeneous inherited neurodegenerative disorders characterized by progressive paresis and spasticity of the lower limbs. Although patients often complain of symptoms that cannot be addressed by the involvement of the upper motor neuron, little is known about the involvement of the peripheral nervous system (1).

Objectives: We aimed to study peripheral nerve excitability in the median nerve of patients with HSP.

Methods: Nerve excitability studies were performed on motor axons by using multiple excitability protocol, TRONDXM4 (QTRAC version 8.2©Professor H. Bostock, Institute of Neurology, London) (2). The median nerve of 26 patients with HSP and 21 healthy controls was stimulated at the wrist with the cathode positioned over the skin crease and anode 10 cm proximally over the lateral forearm. Stimulation was computer controlled and converted to current using an isolated linear bipolar constant current simulator. The following axonal excitability parameters were measured: (i) strength–duration time constant, rheobase; (ii) threshold electrotonus (TE) recorded with sub-threshold depolarizing currents and with hyperpolarizing currents; (iii) hyperpolarizing current-threshold relationship (I/V) calculated from polarizing current between −50 and −100%; (iv) recovery cycle parameters including the relative refractory period, superexcitability and late subexctiability (2,3).

Results: The group comparison (patients vs. healthy controls) revealed abnormalities in the threshold electrotonus with an increase in the depolarizing current, but also in recovery cycle with a delay of the superexcitability and subexcitabilty period.

Discussion and conclusion: HSP is not only characterized by loss of upper motor neuron function but also by changes of axonal excitability in the peripheral nervous system.

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CW11 ACCELERATING INTERVENTION TO RESPIRATORY SUPPORT

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Keywords: early intervention, watch-PAT sleep studies, respiratory support

Background: Assessment of respiratory insufficiency in amyotrophic lateral sclerosis (ALS) includes measurement of vital capacity (VC) as percent predicted, together with maximum inspiratory pressure (MIP) and nocturnal pulse oximetry (NPO). Previous surveys of nocturnal respiratory dysfunction in ALS patients have reported prevalence of abnormalities in NPO ranging from 3.1% (1) to 11% (2) to 40% (3,4) to 56% (5) to 65% (6). Patient selection and numbers of patients studied were offered as reasons for the differences. In one unselected study of 261 newly diagnosed ALS patients 45/261-17.2% (7) patients with VC and MIP in normal range had abnormal NPO.

Objective: Prospectively evaluate the prevalence of respiratory insufficiency in ALS patients at initial encounter referred to an ALS Multidisciplinary Clinic at an academic medical center in the Southeastern USA.

Methods: Patients (Group1-49) with low VC, low MIP or respiratory symptoms received volume-cycled non-invasive ventilator (V-NIV) treatment recommendations at the first clinic visit from 2011-2014, which occurred at various times post disease onset. Patients (Group 2-93) who had higher VC, higher MIP and minimal respiratory symptoms underwent home-based nocturnal pulse oximetry in the context of Watch Peripheral Autonomic Tomometry (WatchPAT) testing to provide more substantive analyses of apnea and sleep episodes (8).

Methods: Group1 Patients (29M; 20F) had significantly lower VC-%predicted (66.5-95% CI(58.7-74.2); P=0.012) compared with Group 2 patients (61M: 32F) who required WatchPAT testing (77.2-95% CI(72.6-81.8)). Group1 patients started V-NIV treatment based on VC criteria (17/49-34.7%) and MIP or other criteria (22/49-44.9%) while some patients did not start V-NIV for various reasons (10/49-20.4%). Group 1 patients started V-NIV based on VC criteria (13/93-13.9%) and MIP or other criteria without need for WatchPAT data (8/93-8.6%). An additional set of patients (16/93-17.2%) from Group2 demonstrated nocturnal hypoxemia or other sleep abnormalities on WatchPAT testing and were started on V-NIV while other patients (56/93-60.2%) in Group2 did not meet the these criteria for V-NIV intervention at the intake encounter. When V-NIV deployment initiated, it was required by 80% of patients in both Group1 and Group 2 patients, by 42 months after disease onset.

Discussion and conclusion: WatchPAT testing accelerated the appropriate deployment of V-NIV in patients with ALS sleep-disordered breathing (16/37-43.2%) consistent with earlier reports (7).

Conclusions: WatchPAT testing permits a simple, feasible means of assessing ALS sleep-disordered breathing early in the course of ALS and affords the opportunity to provide additional information to support early respiratory support for these patients.

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CW12 ALS AND AIRWAY CLEARANCE (ALSAC): IS THERE A BEST THERAPY FOR AIRWAY CLEARANCE IN PATIENTS WITH ALS?

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Keywords: airway clearance therapy

Background: Breathing and airway clearance is essential for ALS patients. As the disease progresses, coughing becomes ineffective and aspiration occurs leading to

respiratory insufficiency/failure, which can lead to death. AAN recommends the mechanical insufflator/exsufflator (MIE) as the effective airway clearance therapy (1). However, there is no strong evidence to support using the High Frequency Chest Wall Oscillator devices (HFCWO) for airway clearance. Research on using MIE and HFCWO together is limited. It is important to provide appropriate therapeutic intervention for airway clearance therapy as soon as patients have difficulty mobilizing secretions.

Objectives: The purpose of this pilot study was to examine the use of MIE and HFCWO individually and in combination as effective interventions for airway clearance over 6 months.

Methods: Patients and their caregiver (PAC) were randomized into one of these three intervention groups. The PAC completed perception of problem and quality of life surveys. A respiratory therapist taught the PAC to properly use the devices following a standard protocol and to document compliance, dyspnea, secretion amounts and medical events. The PAC had clinic visits every 3 months and completed global impressions of change in secretion removal (GIC), perception of problems and quality of life surveys.

Results: Over two years, 28 PAC were randomized: 3 patients (1/group) died during the study, 13 dropped out and 12 PAC completed or are active. The 12 participants include 6 females and 6 males; 8 Caucasian, 3 Hispanic and 1 Black with a mean age of 62 years (range 31-76). The GIC between beginning the intervention and the second and third visits was slightly better or not any different as rated by both the patient and caregiver. The Respiratory Complications Severity Scale demonstrated 3 had doctor visits for respiratory complications and 7 had no respiratory complications.

Discussion and conclusion: Findings from this study suggest timing of diagnosis and initiating use of these devices and compliance with these interventions impact the disease outcomes associated with airway clearance. Further research is needed to determine best practices for when to order the airway clearance devices, patient use compliance and caregiver support. Undoubtedly, it is the goal of practitioners to support the use of these interventions for ALS patients with airway clearance issues thus influencing the quality of life of the patient and caregiver.

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CW13 UPPER MOTOR NEURON DIAPHRAGM PARALYSIS IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: diaphragm pacing system, diaphragm paralysis, respiratory failure

Background: Diaphragm pacing system (DPS) is used to extend survival in amyotrophic lateral sclerosis (ALS) patients with respiratory failure. Candidates for DPS must have a diaphragm that can be electrically stimulated. Absence of diaphragm movement on the fluoroscopic sniff test (FST) should not exclude patients from DPS as this may occur from upper motor neuron (UMN) disease.

Objective: To report a favourable outcome of DPS in a patient with ALS who had nearly complete diaphragm paralysis on FST.

Case Report: A 50-year-old woman with bulbar onset ALS was evaluated for consideration of DPS surgery. She began non-invasive ventilation 1 month earlier and used this successfully 8 hours per night. The FST showed complete absence of motion on the left and slight inferior motion on the right. Phrenic nerve studies showed normal amplitude responses bilaterally. At DPS surgery, both hemidiaphragms showed vigorous contraction to electrical stimulation. She tolerated DPS surgery and combined gastrostomy tube placement without complications. Two months before DPS the sniff nasal inspiratory pressure (SNIP) was -36. The SNIP values at 2 and 5 months after DPS were -49 and -52.

Discussion and conclusion: Nearly complete absence of diaphragm motion on the FST should not exclude patients from consideration of DPS therapy. This patient demonstrated a UMN pattern of diaphragm paralysis with preserved lower motor neuron diaphragm innervation. At the time of DPS surgery, both diaphragms showed good contractions to electrical stimulation. The patient tolerated the surgery and demonstrated improved respiratory function.

CW14 OUTCOMES OF AMYOTROPHIC LATERAL SCLEROSIS PATIENTS WITH PERCUTANEOUS ENDOSCOPIC GASTROSTOMY AND INVASIVE VENTILATION (TRACHEOSTOMY)

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Keywords: PEG and tracheostomy

Background: Several studies have shown that malnutrition and weight loss are negative prognostic factors in patients with ALS.

Objectives: To analyse pre and post-interventional complications and survival after PEG and invasive ventilation in ALS patients.

Methods: We have examined 500 ALS patients between 2000-2014 years retrospectively.70 of 500 ALS patients under went to PEG and invasive ventilation during this time. FVC weight loss and dermographic data were assessed. Complication rates and overall survival rates were analyzed.

Result: There were 35 women and 35 male the ALS patients, with PEG and invasive ventilation. The age interval was 23-82 years (Mean age was 56.6). The survival time range was from 3 hours -12 years after intervention.

Discussion and conclusion: In these case series PEG tube and non-invasive ventilation (tracheotomy) were minimal pre and post procedure complications. Our results suggest that tracheotomy and PEG placement may improve outcomes even in ALS patients with severe respiratory impairment.

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CW15 SPEECH AND LANGUAGE THERAPY AS A RESOURCE IN THE CREATION OF PERSONAL LEGACY BOOKS

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Keywords: narrative, memory books, dysarthria

Background: Narrative theory is based on the premise that a patient should tell the story of their illness in their own words. Communication disability, secondary to MND contributes to a patient's illness experience as well as limiting their ability to construct a narrative to describe that experience. Developing memory books to describe ones life and illness is a way of maintaining a sense of personal worth as well as creating a legacy to leave behind for loved ones. Communication disability can limit a person's involvement in such projects especially when it co-occurs with significant physical disability. Speech and Language Therapy (SLT) as a profession have a role to ensure equal access to such projects for patients with communication disability.

Objectives: SLT acted as a resource to facilitate a woman with MND who presented with anarthria and complete loss of limb movement to produce memory books for her young children. These books were to be used to support the children through grief counselling.

Method: The SLT department recruited a graduate SLT for an eight week project to facilitate this patient to write stories for her children. The patient used eye movements to make Yes/No responses to letter selection on a partner-assisted alphabet chart and to type information on an eye gaze controlled electronic communication device. The focus was to ensure that this was a patient-led process with the SLT graduate, supported by the SLT, to facilitate rather than lead the project.

Results: A separate memory book containing a personal letter, photographs and stories of pregnancy, birth and childhood was created for each child. This was achieved in full via the patient using her eye movements alone to spell words using communication aids. Throughout the sessions with the graduate SLT, it became apparent that the process of telling her stories to a communication partner brought its own rewards in terms of affirming her identity.

Discussion and conclusion: Although the target was to work towards the end product of a memory book for each child, the process of story telling appeared to improve this patient's well being and aid her acceptance of her limited life expectancy. Her feelings of personal worth and sense of control were also reaffirmed. The creation of memory books is a well utilised tool in supporting children of cancer patients through anticipatory grief. From the success of this project, it appears to be a tool that should be further explored for people diagnosed with MND who are also parents of young children. SLT has a role to play in facilitating the subset of this group who also have significant communication disability.

CW16 TREATMENT OF NEUROGENIC STUTTERING IN MND: A CASE REPORT

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Keywords: speech, stuttering, treatment

Background: In addition to dysarthria, other communication disorders such as progressive apraxia of speech (1) and aphasia-like impairments (2) have been described in patients with ALS. In comparison, there have been very few descriptions of neurogenic stuttering (NS) in MND (4). NS is defined as dysfluent speech that is acquired as a result of CNS damage. Dysfluencies consist of blocks, repetitions and sound prolongations.

Objectives: To describe a case of MND with neurogenic stuttering and report response to a short-term fluency intervention.

Methods: Chart review; patient interview; analysis of pre- and post-fluency treatment speech samples; self-report questionnaires; individual treatment sessions (one

hour bi-monthly for three months) focusing on fluency techniques and counselling regarding attitudinal/behavioural components of stuttering.

Case Report: 61 year-old man who started experiencing recurrent falls due to reduced balance in 2000. Speech changes were first noted in 2002. He was diagnosed with PLS in 2004. At the time of diagnosis, he presented with a moderate dysarthria dominated by spastic features. Other symptoms included mild dysphagia, spasticity in all four limbs, reduced fine motor dexterity, decreased balance and limited endurance. In 2006, he developed significant stuttering which, in combination with his dysarthria, rendered his intelligibility poor. In 2013, the patient was referred to the adult fluency clinic of the McGill University Health Centre as it was felt that the main obstacle to intelligible speech was stuttering rather than dysarthria. Gains in both speech fluency and attitudinal/behavioural components were observed after fluency therapy. Speech samples showed improvements: percent syllables stuttered in spontaneous speech went from 23 to 14.7, severity rating diminished from 8 (severe) to 5 (moderate) and rate of syllables per minute increased from 29 to 77.5. Average and maximum duration of dysfluencies were also notably reduced. Improvements in reported self-confidence, avoidance, and openness were also made. As per subsequent email exchanges with patient, fluency gains were maintained for over a month after the completion of therapy.

Discussion and conclusion: Short-term fluency therapy was beneficial in improving speech in a patient with MND associated with dysarthria and severe neurogenic stuttering. Typically most intervention in speech therapy for patients with MND is targeted at teaching compensatory strategies to manage worsening dysarthria and to implement various augmentative and alternative communication strategies. However, when stuttering also presents a significant barrier to functional oral communication, it may be worthwhile for patients to learn fluency enhancing strategies to maximize their potential for natural speech as long as possible.

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CW17 A PILOT STUDY ASSESSING A NEW EYE-WRITING DEVICE ALLOWING CURSIVE WRITING WITH SMOOTH PURSUIT EYE MOVEMENTS IN SUBJECTS WITH ALS

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Keywords: pilot study, communication tool, eye-writing device

Background: Motor weakness progression in ALS challenges communication modalities such as writing or speech with a marked impairment of quality of life. In recent years the development of appropriate communication tools played a key role maintaining patients in an efficient interaction with environment and caregivers. However there is a need for tools to customize communication and provide a creative space. Eye on-line (EOL) is a new communication device with which the user is presented with an illusion inducing visual stimulus resulting in the perception of illusory movement that can be followed by the eye, so that smooth pursuit eye movement can be sustained in arbitrary directions (1). After an appropriate training participants gain volitional control over smooth eye movements and can generate digits, letters, words or drawing at will.

Objectives: The primary objective of the study is to assess the feasibility of the use of EOL device in ALS patients. The secondary objective is to assess its clinical safety in subjects with ALS. We added exploratory objectives to evaluate eye movements in ALS patients, to study factors (neuropsychology, eye movements) that may influence the use of the apparatus and to evaluate a Bayesian computational model for online character recognition (2).

Methods: Eighteen subjects with ALS and motor impaired normal writing will be recruited for duration of participation of four weeks per patient. The intervention will consist in a training program to the device during six visits on site allowing a gradual acquisition of the ability to perform an eye-writing. The primary endpoint is the recognition by an outside observer of the digits 0-9 produced by the patient with the device. The secondary criteria is the record of adverse events and serious adverse events occurred during the study other than those directly related to ALS.

Discussion and conclusion: EOL device potentially offers a creative and personal means of linguistic and emotional expression in subject with motor disability. The study has been open for patient recruitment since June 2014. Results are expected in mid 2015.

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CW18 PREVALENCE OF PERIPHERAL OEDEMA IN ALS AND CURRENT TREATMENT GUIDELINES

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Keywords: peripheral oedema, swelling, management of symptoms

Background: Oedema is a palpable swelling produced by expansion of the interstitial fluid volume, as a result of imbalance in filtration of fluids in distal limbs back into circulatory system. This occurs for a multitude of reasons including kidney, heart and liver disease and medications, to name a few. Occasionally, peripheral oedema is seen in patients with ALS due to immobility and increased fluid pressure from venous stasis, which renders currently available treatments such as loop diuretics and sodium restriction useless in controlling swelling and preventing further damage to the veins and tissue in PALS. Current review of literature shows that research directed treatment guidelines of oedema in ALS are scarce at best.

Objective: To determine the prevalence of oedema in ALS patients seen in a New York ALS clinic and survey current management strategies used by patients and physicians.

Methods: 100 charts of PALS were reviewed to assess for prevalence of peripheral oedema. Methods of management and their success, if any, were recorded.

Results: Review of 100 charts over course of 4 months showed a prevalence of 18%. The site of oedema corresponded to weakness, with swelling in fingers seen with flail arms and ankle swelling seen with leg weakness and immobility. Variety of treatment modalities were suggested and tried by patients, including elevation, compression stockings and massage, leading to mixed and non sustainable results.

Discussion and conclusion: Swelling in the upper and lower extremities, due to immobility and muscle weakness, is a common finding in patients with ALS. This research was aimed at surveying the prevalence of oedema and common strategies for management used by patients and physicians. The review of charts has shown an 18% prevalence of oedema in PALS, with a variety of treatment modalities, with mixed results. Many chart notes did not indicate evaluation of oedema, suggesting incidence could be higher. Currently there are no standardized effective treatment guidelines for management of oedema in this patient population. Further research is needed to develop a more standardized approach to assessing and treating ALS related peripheral oedema.

CW19 THE LOST ART OF KISSING

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Background: While ALS may not affect sexual function directly, it can affect self image, mobility and breathing to an extent that impacts sexual activity for patients. Additionally, one's sexual partner is suddenly placed in role of a care giver, possibly diminishing intimacy due to this change. This presents an area of need for patients and their partners that could be addressed at clinic but is rarely a covered topic at the visits.

Objective: To study the extent to which intimacy is affected by ALS, PALS and partners comfort level expressing their concerns at clinic, substitution methods used for displaying intimacy, level of satisfaction with support provided in clinic, and areas of improvement for the clinic team.

Methods: A questionnaire was distributed to patients and partners over 6 month, assessing if PALS or partners had perceived changes to their sexual relationship since diagnosis, if they discussed this with their partner, or with anyone on the clinic team, what substitute methods they use if any, and has or how could the clinic team approach their concerns. An additional questionnaire was given to the team members, assessing their perceptions of intimacy in PALS, comfort in discussing this with patients and availability of information to provide.

Results: At the time of submission, 14 patients were interviewed. All reported drastic decline in sexual activity since diagnosis, some substituted hand holding and hugging in place of intercourse. None had previously been approached by anyone in clinic to discuss this topic and all were open to talking about their concerns with any qualified team member.

Discussion and conclusion: Evidence suggested that sexuality is a defining element in quality of life and is strongly affected by diagnosis and symptoms of ALS, for both patient and their partner. Due to limited understanding and sensitive nature of topic of sexuality, few studies have been conducted to address this and PALS' concerns on matters of intimacy are often avoided by health care practitioners. Addressing these issues can help bring the couple closer and enable a better quality of life. The aim of this survey was to analyze the effect of disease process on intimacy, and how well these concerns are addressed. We were able to show that in our patient population, sexuality and intimacy is indeed affected by ALS, and that patients are open to discussing this with qualified clinic staff. Lack of information to provide to patients, regarding positioning and safety, was found to be the main barrier felt by clinic staff which limited their discussion with patients.

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CW20 EXPLORATION OF CAREGIVING EXPERIENCES IN AMYOTROPHIC LATERAL SCLEROSIS: A PRELIMINARY ANALYSIS

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Keywords: caregiver experiences, difficult, positive

Background: Management of ALS is palliative, aimed at maximising quality of life and minimising the burden of disease for patients and caregivers. Caring for a person with ALS can be an unrelenting commitment in a situation of rapid and continuous change with time spent providing care increasing across the illness trajectory.

Objectives: To explore positive and negative aspects of the caregiving experience for informal caregivers of ALS patients in Ireland.

Methods: This analysis is part of a longitudinal study of 100 people with ALS, and their primary caregiver recruited from those attending a multidisciplinary ALS clinic in Dublin. As part of a structured interview caregiver burden is assessed through the Zarit Burden Interview (ZBI), while a number of open-ended questions explore their experiences of caregiving. Caregivers are asked to outline what would help them at the moment, and what for them, are some of the difficult and good things about caregiving. Thematic analysis is used as a qualitative descriptive approach to identify, analyse and report themes from the open-ended questions, and considers both the latent and manifest content of the caregiver responses.

Results: To date information has been collected on 53 informal caregivers. This group is predominantly (68%) female, with the majority of caregivers spouses/partners of ALS patients. The average number of hours of care provided is 50. The mean ZBI score is 27.36 indicating 'mild to moderate burden' (1). In relation to experiences of caregiving, issues identified include requirements for support from family members and health and social care services, fear of the future, frustration, time and social restrictions, changes in identity and roles, and issues around own physical and emotional health. Some positive aspects of caregiving identified include spending time together, closeness and companionship, meeting an obligation, being able to help as long as possible and provide some quality of life to their loved one.

Discussion and conclusion: Caregiver's physical, mental and emotional health influences their ability to provide care to patients with ALS. There are unique and common challenges faced by informal caregivers which translate into different caregiving experiences.

References:
1.Hebert R, Bravo G, and Preville M Canadian J Aging 2000; 19: (4): pp494-507.

Acknowledgements: The project is funded by the Health Research Board (HRB) Dublin as part of the HRB Interdisciplinary Capacity Enhancement Awards.

CW21 CAREGIVER BURDEN IN AMYOTROPHIC LATERAL SCLEROSIS (ALS): A PROSPECTIVE ANALYSIS

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Keywords: prospective, burden, caregiver

Background: A considerable amount of care for those with amyotrophic lateral sclerosis (ALS) is provided by family and friends. Given the multifaceted and progressive nature of the condition this can result in significant caregiver burden.

Objectives: The aim of this study is to identify determinants of burden in caregivers of ALS patients in Ireland.

Methods: This prospective analysis forms part of a larger longitudinal study, tracking 100 ALS patients and their primary caregiver attending a multidisciplinary ALS clinic, over the course of the illness. A structured questionnaire administered to the patient and caregiver collects information on a range of demographic and socio-economic factors, while standardised measures are used to assess caregiver burden (Zarit Burden Interview - ZBI), anxiety and depression (Hospital Anxiety and Depression Scale - HADS), and quality of life (McGill Quality of Life (MQoL) and Schedule for Evaluation of Individual Quality of Life-Direct Weighting – SEIQoL-DW) of both the patient and caregiver. Other variables recorded include patient factors such as site of disease onset, duration of illness and physical function (ALS Functional Rating Scale Revised - ALSFRSR). Patient's clinical details are obtained from their medical charts.

Results: To date, data has been collected on 53 caregivers, the majority of whom are female (68%), spousal caregivers (74%), and living with the patient (83%), with an average age of 56 years. These caregivers spend an average 50 hours a week providing care. The mean Zarit burden score is 27.36 indicating 'mild to moderate burden' (1). There is little difference between males and females in the total burden score, although there is some evidence of higher burden among those caring for a parent rather than a spouse. In general, carer burden increases with hours of care provided and worsening of patient disability, while caregiver quality of life decreases with increasing care burden.

Discussion and conclusion: This study will further examine the determinants of caregiver burden among those caring for someone with ALS over the course of the illness. Factors considered will include: caregiver age; sex; hours of care provided; type of care provided; self-assessed health; depression and anxiety; quality of life; and also patient factors such as cognitive and behavioural status; site of disease onset; duration of illness and physical function. Identification of determinants of caregiver burden will help to target caregiver interventions.

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Acknowledgements: The project is funded by the Health Research Board (HRB) Dublin as part of the HRB Interdisciplinary Capacity Enhancement Awards.

CW22 PSYCHOLOGICAL PROBLEM OF CHILDREN WITH PARENTS WITH ALS

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Keywords: younger caregiver, psychology, life style

Background: What the ALS patients and their family need most is information in relation to the disease and the PAS (Personal Assistance Service). Considering most ALS patients are over 40s, their children could be young. The young children should take on most of responsibilities for caring their parents with ALS, whilst also preparing for their own future.

Objectives: This research shows the psychological problem of children caused by caring their parents with ALS.

Methods: Based on a news article about Lee, 10 years old, who cared for her divorced father with ALS, and semi-instructed and in-depth interviews with Kim, 30 years, who had spent her 20s time caring for her divorced mother with ALS. This research studied what kind of psychological problems are there to children with ALS parents.

Result: Being based on a report, Lee's psychological condition is as conjecture: 1) Wants to have her own space and enough time for sleep; 2) She cannot eat anything when Lee thinks of the patient (her father); 3) She really loves the patient, although nursing is ever more difficult.

According to a survey Kim's psychological condition is as follows: 1) Kim has to nurse after finishing part-time job. PAS only give about 3hours per day. She feels depressed every night when she back to home because of the lack of time to get that services that take her time away; 2) She struggles to find person who can nurse her mother instead of her in her family members. Sometimes she would like to have family member who can nurse; 3) She is worried about the future which is getting a job, marriage and childbirth.

Discussion and conclusion: Although the children with parents with ALS need to spend time to prepare their future, they couldn't have personal time or sufficient rest time. Moreover, there was no one to turn to when they was having a hard time. For the children, the parents with ALS are one and only 'PARENTS' as well as patient that they must take care of. Even though caring for their parents with ALS is hard, because they love their parents, they are willing to take burden above their ability. Thus, this research suggests we need the proper PAS and psychological consulting program to help to make the children with ALS parents prepare for their future as well as care for their parents.

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CW23 ASSESSING PATIENT COGNITION AND BEHAVIOUR IN SPECIALISED ALS MULTIDISCIPLINARY CARE: A STUDY PROTOCOL

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Keywords: multidisciplinary care, cognition, behaviour

Background: Awareness of cognitive and behavioural change in deteriorating conditions informs patient and carer approaches to managing their future needs, and

assists health professionals to facilitate timely decision-making for care (1). This research proposes a means to identify changes in amyotrophic lateral sclerosis (ALS) patient cognition and behaviour, and determine their impact on patient psychological wellbeing and caregiver burden.

Objectives: The study will evaluate the feasibility of an assessment package developed for use in specialised ALS multidisciplinary clinics, and the contribution the resulting information makes to patient care.

Method: The project examines the following research questions: 1) How can cognitive and behavioural changes associated with 'ALS plus' be detected in the specialised ALS multidisciplinary clinic setting? 2) What is the impact of cognitive and behavioural change on patient wellbeing and caregiver burden? 3) How do the results of 'ALS plus' assessments contribute to patient care?

The project will be being conducted during 2014 - 2015, as a mixed method study in two phases. Phase 1 is the implementation of the cognitive and behavioural assessment package. Phase 2 is the evaluation of the feasibility of the package. Data collection will take place in three ALS multidisciplinary clinics. Participants are ALS patient and carer pairs, and health professionals. Components of the assessment package are: Addenbrooke's Cognitive Examination-Revised (ACE-R) (2); McGill Quality of Life Questionnaire-Single Item Scale (MQOL-SIS) (3); Kessler Psychological Distress Scale (K10)(4); Motor Neuron Disease Behavioural Scale (MiND-B) (5); and the Caregiver Burden Inventory (CBI)(6).[6]

Conclusions: Psychological and social factors play an important role in ALS patients' wellbeing and quality of life (7). Patient cognition and behaviour should be as comprehensively and proactively assessed as physical status, to identify early changes and provide holistic care. The assessment package seeks to meet these aims, and address a significant deficit in ALS patient care.

Information gained from the assessment supports patient and caregiver understanding of ALS, and promotes their engagement in decision-making for symptom management and quality of life (8). This protocol may have wider application to other conditions involving changes to patient cognition and behaviour. The package has the potential to enhance decision-making, improve service planning and management, and influence policy framing.

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CW24 APATHY, EMOTIONAL EXPRESSION AND PSYCHOLOGICAL ADAPTATION IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: psychopathology, coping strategies, emotional regulation

Introduction: Depressive and anxious symptomatology have been described in the literature. However their relationship to apathy, emotional expression and coping strategies remain unknown.

Methods: The study participation has been proposed to patients, hospitalized for one day. All patients who agreed to participate have signed a written informed consent. All included patients received a booklet with different self questionnaires. These questionnaires assessed the depressive symptomatology (BDI, HAD), the anxious symptomatology (STAI-T, HAD), the positive and negative emotions, (EPN-31), the coping strategies (CHIP Neuro) and the apathy (Marin). The socio-demographics and clinical variables were taken from patient's files.

Results: 150 questionnaires have been collected. The presentation of the results will describe the scores at each psychological scale, and the links between the different studied dimensions (anxiety, depression, apathy, expressed emotions and coping strategies). Thus we will present results according to the severity and survival. We will present the variables which seem to be related to the apparition of anxious or depressive symptoms.

Discussion and conclusion: Discussion will be focused on the possibility to identify in ALS patients some vulnerability to psychopathological symptoms, and their relationship with the clinical variables of the disease in order to identify which patients should benefit from a psychological intervention.

CW25 CHALLENGES AND REWARDS OF SETTING UP A NETWORK MODEL: SOUTH

WALES MOTOR NEURONE DISEASE (MND) CARE NETWORK

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Keywords: care network, integrated care, multi-disciplinary team

Background: The network model involves building on established links between key professionals from a variety of specialities and professions and forging new links across primary, secondary and tertiary care, and across health and social care, community and hospital settings. Optimising the care of people with MND (pwMND) is central to the model and is being delivered through the planning, delivery, implementation and evaluation of local multi-disciplinary team (MDT) clinics within each health board. In this poster presentation we outline the process of delivering the network model of care, its challenges and rewards.

Methods: In order for the network to meet the needs of people with MND in South Wales initial work consisted of: Obtaining details of all pwMND in South Wales subject to their consent; Identifying names of professionals and lay persons involved in their care; Identifying those professionals with particular interest in MND and developing links between them; Conducting a baseline audit of care via pwMND; Delivering 2 network study days to develop interest and up-skill; Setting up MDT's and MDT clinics; Highlighting existing problem areas; Identifying the drivers of key stakeholders and planning and focussing on outcomes early in the process.

Results: We have identified all pwMND in South Wales who are known to NHS services and have an agreed referral pathway. We have identified interested and engaged professionals and have established local MND clinics. Terms of reference have been developed for the clinics to include agreed standardised assessment, outcome and assurance methods. The main problem areas identified have been inequity of access to NIV and cough assist, and timely and appropriate decisions and action on gastrostomy tubes, equipment and adaptations.

Discussion and conclusion: The challenges of setting up this model of care include familiarising with and negotiating differing service configurations across all health boards and social services providers, engaging and up-skilling health and social service staff, practical considerations of clinics with multiple staff and pwMND, and negotiating input to clinics with service managers.

There are many rewards from setting up this model of care, including the satisfaction of providing holistic seamless and integrated patient care which is efficient, effective and timely, better access to information and support for pwMND who have given extremely positive feedback, better peer support and more effective communication between professionals. By using local services team members bring differing knowledge and skills allowing for cross fertilisation of ideas between clinics which feeds development of the network. The network has excellent links with local MND Association branches and Association Visitors which enhances its development.

The Network approach to providing care for pwMND can be helpful and rewarding with many benefits for pwMND, their families and professionals.

Acknowledgements: Motor Neurone Disease Association; All members of the MND MDT's in South Wales.

CW26 REVERSING THE HUB AND SPOKE MODEL: PATIENT EXPERIENCES OF THE DEVELOPMENT OF A COMMUNITY BASED MULTI DISCIPLINARY MOTOR NEURONE DISEASE (MND) CLINIC TO MEET THE MOTOR NEURONE DISEASE ASSOCIATION 'STANDARDS OF CARE'

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Keywords: community, hub and spoke model, patient experience

Background: Specialist MND services in the UK often adopt a hub and spoke model with regional acute centres coordinating services - offering specialist advice, guidance and support to local community teams.

In the London borough of Barnet, acute hospital investigations, diagnosis and support may be delivered via four different acute NHS Trusts, however all patients are managed by one community team - the Community Neurological Conditions Management Team (CNCMT). It was identified by the CNCMT that there had been an increase in complex case management of pwMND by the team and reports by patients of dissatisfaction due to fragmented service provision and long waits for community team intervention.

Following the development of a part time MND coordinator post, a community based MDT clinic was launched to improve patient experience and meet best practice guidelines (1, 2). The MDT clinic and community service provision thereby sits as the 'Hub' of the model, supporting specialist acute 'Spoke' partners to deliver seamless care to patients. pwMND are invited to attend the clinic with direct access to the MND coordinator and community team therapists for triage, assessment, advice and signposting.

Objectives: To evaluate patient and carer experience of a community based multi-disciplinary MND clinic and to identify improvements in clinical delivery of services.

Method: An audit of the community service was undertaken using the MND Association Standards of Care checklist pre- and post clinic launch. In addition, patient experience is being measured in a 16 point questionnaire using a 5 point Likert scale.

Results: The MND Association audit demonstrated improvements in six out of twelve key areas. Most notable of these improvements were offering patients an appointment within two weeks of diagnosis and improving communication across agencies. Twenty-nine patients with MND were seen in the clinic (thirteen outpatient, sixteen home visits) since clinic launch in April 2013, and patient experience is currently being collected and due to be reported on in July 2014.

Discussion and conclusion: The current service provision in Barnet for people with MND was audited and identified significant gaps in comparison to best practice. Traditionally large acute hospitals have coordinated care for people with MND; however it is felt that for rapidly progressing conditions, a local service accessing expert advice when required from the acute sector provides a better patient experience. The development of a community based MDT clinic aims to improve the patient pathway throughout disease progression. It is hoped that changes to service delivery will demonstrate a positive relationship to patient experience alongside better coordination and communication between partner organisations.

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CW27 OCCUPATIONAL THERAPY- MAINTAINING FUNCTION THROUGH THE LIFE SPAN

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Keywords: occupational therapy, adaptive equipment, multidisciplinary care

Background: Little research exists to show the value of the Occupational Therapist (OT) as a multi-disciplinary team member, although published guidelines state that it is a recommended service for persons with ALS/MND. Many physicians and certainly the public have a lack of understanding of the vital assistance the OT can bring to the table, and many multidisciplinary clinics do not have their own OT.

Objectives: To educate the ALS/MND community about specific ways that the Occupational Therapist can increase the function, independence and quality of life of the person with ALS/MND and subsequently, their caregivers.

Methods: Information was collected from ALS/MND patients about what adaptive equipment, education and services they have received from OT, and how those items affected them functionally. Information regarding quality of life and how having those OT services affected them was also collected.

Results: OT is seen as a valued service by the ALS/MND patients at our ALS clinic, for the resources, training, education, equipment and ideas they offer.

Discussion and conclusion: OT can assist persons with ALS/MND throughout the disease process, and it is important that Physicians, Community staff, Nurses, outpatient and home health clinicians and the patients and families themselves are aware of the need for OT throughout the course of the disease.

OT is a valuable multidisciplinary team member, and should see every person with ALS/MND in the clinic, home and community throughout the lifespan.

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CW28 QUALITY OF LIFE IN AMYOTROPHIC LATERAL SCLEROSIS: A REVIEW

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Keywords: quality of life, measurement, caregivers

Background: Given the debilitating nature of Amyotrophic Lateral Sclerosis (ALS), patient care is largely focused on relieving symptoms and maintaining quality of life (QoL). There has been a significant amount of research into the concept of QoL and its measurement in ALS patients.

Objectives: This review aims to identify themes associated with quality of life in patients with ALS and their caregivers.

Methods: Relevant themes were identified through a literature search between January and May 2014 using the databases PubMed and Medline. Additional articles were identified from reference sections listed in the literature found. The search words used were 'Amyotrophic Lateral Sclerosis', 'Motor Neuron Disease', 'Quality of Life', and 'Quality of Life Measures'.

Results: Three major themes were identified from the literature: 1) factors associated with patient QoL; 2) factors associated with caregiver QoL; 3) the measurement of QoL in ALS.

Discussion and conclusion: There remains no overall consensus on the best instruments for measuring QoL in ALS. Both generic and ALS-specific QoL instruments have been used; some as outcomes measures for therapeutic interventions, others for measuring patients' individual contexts. It is important to note that different results on patient QoL can be obtained depending on the measure employed. Health-related QoL instruments, which focus on patients' physical, functional, and emotional status, can be deemed more appropriate for experimental research into therapeutic interventions; while global QoL instruments which take into account non-health related factors (ie psychological, supportive and spiritual factors) seem more informative in analysing patient services and experiences (1, 2).

Specific illness characteristics, such as pain or disability, have been shown to affect QoL in patients with ALS. However, there is often little change in patient QoL despite marked deterioration in overall health and physical functioning. Non-health related factors, such as psychosocial support or religion, have been shown to help maintain patient QoL in spite of disease progression. There is a misconception of ALS patients' QoL being greater than perceived by caregivers. Lower caregiver QoL scores compared with that of patients reflects the possible burden caregivers can experience with this condition. Caregivers' QoL is negatively affected both by patient physical symptoms, such as respiratory function and pain, and behavioural changes, such as disinhibition and impulsivity.

Acknowledgements: This study is funded by the Health Research Board (HRB) Dublin as part of the HRB Interdisciplinary Capacity Enhancement Awards.

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CW29 MISCONCEPTIONS IN PALLIATIVE CARE FOR PATIENTS WITH ALS

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Keywords: palliative care, quality of life

Background: Amyotrophic Lateral Sclerosis (ALS) is a devastating terminal neurodegenerative disease that follows a relatively predictable clinical trajectory, as such palliative care consultation should begin soon after diagnosis. Previous studies found in a randomized control trial that 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 of care (1). 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.

Objectives: The purpose of our study is to address and measure, by qualitative analysis, common patterns in patients and caregivers' misconceptions of palliative care and ultimately determine when patients feel most comfortable addressing treatment plans and future directives in a clinical setting.

Methods: A questionnaire containing 12 questions will be administered to patients and their caregivers during their clinic visit to assess their comfort in addressing future concerns with the clinic team and level of general understanding of the goals of palliative care. We plan to analyze their responses to review trends in the knowledge patients and their caregivers have of palliative care and future clinical planning, and address how these responses can lead to further changes in our methods of care delivery. This feedback will help us evaluate the quality of care delivered by our clinic team regarding advanced planning and future directives, as well as a tool for understanding when palliative care discussions should be initiated for patients and educating them on the true meaning of palliative care.

Discussion and conclusions: Because complex illnesses such as ALS, thrusts patients into a decentralized and often confusing healthcare system, palliative care seeks help from many sources typically found in multidisciplinary clinics. By addressing common misconceptions in palliative care and seeking answers from patients and their families/caregivers about future planning, we hope to discover when patients ideally want to begin discussions about advanced directives and treatment goals throughout their disease.

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CW30 COLLABORATION BETWEEN ALS AND PALLIATIVE SPECIALISTS IN DENMARK

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Keywords: palliative rehabilitation, collaboration

Background: An interview study conducted by The Rehabilitation Centre for Neuromuscular Diseases (RCfM) in Denmark in 2011 of close relatives to people with amyotrophic lateral sclerosis (ALS) showed that 8 out of 120 patients died in a hospice. In 8 % of the cases, the ALS patient did not die at the desired place of death and the close relative said that in 27 % of the cases, death was unexpected.

The study supports RCfM's experience with the need for intensive, palliative counselling on symptom relief and coping with a lethal disease. Thus, it is crucial to look into possible challenges in referring ALS patients to palliative teams and to identify the type of ALS related information palliative specialists require.

Objectives: To establish formalized partnerships between ALS and palliative specialists in Denmark and to develop a set of evidence-based guidelines to improve the palliative effort in ALS rehabilitation.

Methods: A workshop about palliative services to ALS patients arranged by RCfM and attended by specialized palliative units. Contact to 12 specialized palliative units in East Denmark. Data obtained from 7 units concerning: Experience with ALS patients, application of the questionnaire EORTC QLQ C15, option of home-based care and admissions for symptom relief, collaboration with the primary sector, options for ending treatment with non-invasive positive-pressure ventilation (NIPPV) and invasive mechanical ventilation (IMV), advance directives.

Results: All hospices had limited experience with ALS patients: 5 out of 7 hospices experienced ALS patients more care and resource demanding due to the complexity of the disease; The EORTC QLQ C15 questionnaire is being implemented by all palliative units and data are reported centrally. The questionnaire cannot stand alone, but systematic use will highlight experiences of QoL and the effect of symptom relief; 5 out of 6 hospices offer home-based care and symptom relief admission prior to the terminal phase; All units collaborate closely with the home-health nurse whereas collaboration with the GP depends on his/her commitment; General knowledge and experience with NIPPV and IMV treatment is very limited, although 4 out of 7 want to collaborate with the national respiratory center on legal termination of the treatment. This, however, requires profound knowledge of the patient.

Discussion and conclusions: Visits to the seven units showed limited experience with ALS patients. Specific knowledge about disease progression and personal contact is needed and provision of such knowledge is imperative for future partnerships.

The budding partnership provides the scope for adhering to the recommendations of the European guidelines for ALS that death be addressed as early in the disease course as possible and that clear agreements be made on terminal phase treatment.