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Introduction

This year sees the International Symposium on ALS/MND, organized by the Motor Neurone Disease Association, UK in collaboration with the International Alliance of ALS/MND Associations, return to Australia for the first time in almost a decade. We are welcomed to Sydney by our hosts at MND Australia, in partnership with MND New South Wales.

Under the leadership of their chair Prof Wim Robberecht, the Symposium Programme Committee has compiled a stimulating and varied platform programme. The meeting opens and closes with joint plenary sessions that reflect the emergence of new understanding of disease mechanisms and therapy development. The parallel scientific and clinical sessions go on to explore a wide variety of pertinent themes in more depth, from target pathways, disease models and biomarkers, through to clinical trials, holistic care and the development of practice guidelines. The programme also offers plenty of new perspectives, with key insights from other neurodegenerative diseases, a fresh look at the BMAA story and a truly international offering of care practice findings.

Once again this year, the quality and quantity of the poster presentations promise plenty of lively discussion and debate across the two dedicated poster sessions and beyond, furthering the international exchange of knowledge.

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SESSION 1 JOINT OPENING SESSION

C1 WHAT HETEROGENEITY OF ALS PHENOTYPE MAY BE TELLING US ABOUT PATHOBIOLOGY

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Keywords: phenotype, propagation, pathobiology

Introduction: The marked heterogeneity of motor phenotypes of ALS is well recognized and implies the existence of an underlying unified explanation. In the earliest clinical stages, motor phenotypes are simpler than at later stages because upper and lower motor neuron (UMN and LMN) neuropathology has not summated. At such stages, several observations can be formulated.

Formulation:

- Nearly all patients have focal onset of symptoms, suggesting motor neuron degeneration has a discrete anatomic origination.
- The affected UMN and LMN both share the same body region, suggesting the synaptic connectivity between them was important when disease triggered.
- The degrees to which the UMN and LMN are affected are highly variable, suggesting stochastic distribution of the pathogenic factor between them.
- The degree to which the UMN and LMN are affected early in disease seems to remain constant throughout the disease, suggesting no further recruitment between UMN and LMN occurs after disease triggers.
- The progression of UMN and LMN clinical deficits spreads outward contiguously along respective neuronal anatomy. In this regard, the incongruities between the two levels explain the seemingly complex phenotypes. One type of incongruity involves somatotopic anatomy: At the UMN level, for example, the arm area is contiguous to the ipsilateral leg area while at the LMN level, the arm area is contiguous to the contralateral arm area. The other type of incongruity involves anatomic spread distances: The UMN level, organized as laminar sheets, spans 12 cm in each hemisphere while the LMN level, organized as a column, spans 55 cm rostral to caudal. Because of these incongruities, the superimposed clinical effects of a spreading neuropathology become complex-signs of motor dysfunction summate both within and between levels.
- The highly variable rates of progression are independent of the anatomy of the neuropathology. In this regard, it is important to note that progression rate and survival are independent measures of disease: progression rate is largely determined by the kinetics of motor neuron degeneration while survival is determined by this and the anatomy of the neuropathology (distance to spread to respiratory motor neurons and UMN/LMN distribution).

Conclusion: The fundamental defect in ALS could be a focally triggered and outwardly propagating process and thus the molecular biology of propagation could be fundamental in pathogenesis. The fact that a single genetic defect in FALS (even the same mutation in the same gene) causes marked phenotype heterogeneity supports the idea that a single process such as focal propagation could underlie disease. It is also worth noting that frontotemporal dementia, now recognized to be closely related to ALS, has discrete anatomic onsets-left or right frontal or temporal regions-and thus it too can be characterized as a disease with an anatomically spreading pathology.

C2 THE CONTRIBUTION OF GENETIC FACTORS TO SPORADIC ALS/MND

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Keywords: sporadic ALS, genetic penetrance, risk factor

Like most other diseases the majority of ALS/MND subjects occur sporadically in the population where no other affected family members are known. In only about 5-10% of cases is another at risk family member known to have had ALS.

In families with more than one affected individual, all modes of inheritance (dominant, recessive or X-linked) are found, indicating that the disease in these families has a number of different genetic causes. In some dominantly inherited families 50% of at risk individuals eventually develop ALS. In most families only a few members develop the disease, suggesting reduced effect (penetrance) of a weak gene variation which could be regarded as a 'genetic risk factor'. These intermediate penetrance families are part of a spectrum extending from fully dominant disease to sporadic cases.
Dominantly inherited families are themselves genetically heterogeneous and are caused by a number of genes with disease causing variations (mutations). In our clinic, mutations in SOD1, FUS and TDP43 account for nearly all of our fully penetrant families but these families represent less than 30% of all our families. Most recently reported ALS genes, such as VCP and OPTN account for less than 1% of familial cases (or less than 0.1% of all cases). It is therefore likely that the remainder of our ALS families have weakly penetrant mutations in many, as yet undiscovered genes. These gene variations are hard to find. Such weakly penetrant mutations are also a possible cause of sporadic ALS cases. Whether all sporadic cases have weakly penetrant risk factor genes remains to be determined.

This suggests that sporadic ALS will prove to be very heterogeneous with many risk factor genes. Determining whether these interact with each other and environmental factors is a challenge for the future. Hopefully, common mechanisms or a final common pathway leading to motor neurone death will be found, thus opening up prospects for a simple treatment. Because of the heterogeneity of ALS it is possible that specific treatments for different causes of ALS may be required.

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SESSION 2A PATHOBIOLOGY OF ALS/MND

C3 MICROGLIA - T CELL - MOTONEURON DIALOGUES INFLUENCE DISEASE PROGRESSION

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Keywords: neuroinflammation, microglia, T cells

Neuroinflammation is characterized by activated microglia and infiltrating T cells, and is a prominent pathological feature of ALS. Experimental models suggest that activated microglia of the innate immune system and $CD4^+$ T cells of the adaptive immune system contribute significantly to disease progression. In early stages of disease in mSOD1 mice the first response to motor neuron injury appears to be mediated by neuroprotective microglia, while in later stages of disease, this neuroprotective response is transformed into a cytotoxic response. In mSOD1 mice lacking functional $CD4^+$ T cells, disease progression is accelerated, suggesting a neuroprotective role for T cells as well as microglia. In mSOD1 mice with functional $CD4^+$ T cells, $CD4^+CD25^+FoxP3^+$ T cells (Tregs) are increased during the early slowly progressing phase of disease; as the rate of disease progression accelerates, these numbers rapidly diminish. During the rapid phase, the microglia transform into a toxic M1 phenotype with increased mRNA levels of NOX2 and IL-1β, marked decrease of Tregs, and increased numbers of infiltrating effector T cells (Teffs). The passive transfer of wild-type $CD4^+$ T lymphocytes into ALS mice lacking functional T lymphocytes lengthened disease duration and prolonged survival (1). The passive transfer of endogenous regulatory T lymphocytes from early disease stage mSOD mice was substantially more immunotherapeutic, sustaining interleukin-4 levels, increasing Ym1 and CX3CR1 neuroprotective microglia, and further lengthening disease duration and prolonging survival. The stable disease phase was extended by 88% using mutant SOD Treg. Thus, in the experimental model the dialogue among microglia, T cells and motor neurons suggests that the immune response is not merely a consequence of injury, but actively influences and significantly contributes to the balance between neuroprotection and neurotoxicity. It is clear that neurodegeneration is non-cell autonomous, with the innate and adaptive immune systems contributing to neuronal viability and disease progression.

When these observations were extended to ALS patients individuals with more rapidly progressing disease had decreased numbers of regulatory T lymphocytes, and the numbers of regulatory T lymphocytes were inversely correlated with disease progression rates. These data suggest that immunotherapeutic interventions must begin early in the pathogenic process since immune dysfunction occurs at later stages. The cumulative mouse and human ALS data suggest that increasing the levels of regulatory T lymphocytes in patients with amyotrophic lateral sclerosis at early stages in the disease process may be of therapeutic value. A greater understanding of what dictates the presence of cytotoxic or neuroprotective immunomodulation and how to limit cytotoxicity and enhance neuroprotection would help identify additional targets for immune-based therapy in ALS.

Reference

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C4 ELIMINATION OF INNATE IMMUNE SYSTEM ADAPTOR TRIF SIGNIFICANTLY ACCELERATES DISEASE PROGRESSION OF ALS MICE

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Keywords: SOD1, innate immunity, glia

Background: Prior work from us and others indicated that mutant toxicities within microglia and astrocytes accelerated the disease progression of mutant SOD1 mice. Although the recent work demonstrated that acquired immune system is involved in the disease process, the role of innate immune system in motor neuron disease was not fully investigated. Toll-like receptors (TLRs) play a key role in innate immune responses requiring MyD88 (myeloid differentiation factor 88) and TRIF (TIR domain-containing adaptor inducing interferon-β) as essential adaptor proteins for signaling.

Objectives: To determine whether the innate immune system is involved in the disease process of amyotrophic lateral sclerosis.

Methods: The gene expression profiles of lumbar spinal cord from symptomatic mutant SOD1 mice were obtained by microarray approach and subsequently analyzed using cell-type specific transcriptome. The mating experiment was carried out by crossing $SOD1^{G93A}$, $MyD88^{-/-}$, and $TRIF^{-/-}$ mice. The disease onset, duration, and survival of these cohorts were monitored. The mRNA expression levels of cytokines, chemokines, and neurotrophic factors in the lumbar spinal cords from symptomatic stage were determined by quantitative reverse transcription PCR.

Results: Gene expression profile indicated approximately 70% of upregulated genes were derived from microglia and the pathway analysis indicated the involvement of the innate immune pathway. An accelerated disease progression with shorter survival time was seen only in $SOD1^{G93A}/TRIF^{-/-}$ mice (Mean survival time: $SOD1^{G93A}/TRIF^{-/-}$: 138 days, $SOD1^{G93A}$: 162 days, and 50% reduction in disease duration). The duration of disease and survival time of $SOD1^{G93A}$ did not change when MyD88 was eliminated. The expression levels of several chemokines were significantly suppressed in $SOD1^{G93A}/TRIF^{-/-}$ mice as compared with $SOD1^{G93A}$ mice.

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Discussion and conclusions: Although MyD88 is responsible for most TLR-signaling except for TLR-3, MyD88 has a marginal effect on disease course in ALS models. To our surprise, the basal activity of innate immune response through TRIF, which resides downstream of TLR-3 and 4, plays an important role to control disease progression with regulation of several chemokines. Further identification of the misregulated molecules to explain the accelerated disease progression in mutant $SOD1^{G93A}/TRIF^{-/-}$ mice is underway.

C5 AGGREGATED WILD TYPE SOD-1 INCREASES SERUM LEVELS OF CYTOKINES IN PATIENTS WITH ALS

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Keywords: inflammation, SOD-1, disease mechanism

Background: Copper,zinc superoxide dismutase (SOD-1) protein aggregates in neuronal inclusions linked to ALS have toxic properties. They can occur selectively in spinal cord motor neurons in familial and sporadic ALS.

Objectives: We tested the hypothesis that misfolded SOD1 contributes to motor neuron degeneration via inflammatory mechanisms.

Methods: We examined peripheral blood mononuclear cells (PBMC) of patients with sporadic ALS to identify baseline levels of cytokine expression. Next, we studied the cytokine expression in PBMC after they were treated with soluble and aggregated forms of wild type SOD-1. We confirmed the results obtained in PBMC of live patients by studying the infiltration of immune cells in ALS post-mortem spinal cords.

Results: We identified increased levels of several inflammatory cytokines in PBMC of subjects with sporadic ALS, including IL17A, which has previously been linked to Multiple Sclerosis and other immune-modulated disorders, but not ALS. When PBMC were exposed to aggregated forms of wild type SOD-1, the cytokines IL-1β, interleukin-6 (IL-6), and interleukin-23 (IL-23), which induce IL-17A, were elevated. In contrast, the anti-inflammatory cytokine IL-10 was decreased in ALS PBMC. We detected similar results in the anterior horns of ALS subjects, which were infiltrated by macrophages positive for IL-1β and tumor necrosis factor-α macrophages, IL-17A-positive CD8 cells, and IL-17A-positive mast cells. Inflammatory cytokines were induced in ALS macrophages by aggregated SOD-1 through eicosanoid and caspase-1 pathways. In a small subset of patients, the IL-17A levels in PBMC were normalized after treatment with lipid mediators.

Discussion and conclusion: We provide evidence for an inflammatory disease mechanism in ALS. Aggregated wild type SOD-1 protein in ALS neurons could trigger the expression of inflammatory cytokines in invading mononuclear cells, which contribute to the disease progression. Immune-mediating compounds may be of interest in the investigation of novel ALS therapeutics.

C6 ABNORMAL AXOGLIAL COMMUNICATION IN THE CORTICOSPINAL TRACTS COULD UNDERLIE UPPER MOTOR NEURON DEGENERATION IN SPORADIC ALS

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Keywords: corticospinal tract degeneration, demyelination, neuregulin

Background: Sporadic amyotrophic lateral sclerosis (ALS) involves both upper and lower motor systems.Recent observations demonstrate contiguous lower motor neuron disease progression through the spinal column that correlates well with upper motor neuron signs (1). How this progression links the upper with the lower motor system is not known, but could be linked by degeneration within the lateral corticospinal tract (LCST).

Objectives: Here, we have examined the degree of demyelination, axonal loss, microglial activation within the corticospinal tracts of patients with sporadic ALS and linked these to aberrant expression of specific forms of the gliotrophic factor neuregulin1 (NRG1).

Methods: Spinal cords from patients with significant corticospinal tract degeneration and concurrent upper motor neuron signs were compared to patients without significant degeneration as well as controls. The degree of demyelination, axon loss, gliosis and microglial activation were measured. Gene expression (by qPCR) and protein localization of NRG1 isoforms were determined.

Results: Patients who have significant degeneration of the LCST had more pronounced upper motor neuron signs clinically. These patients showed marked demyelination and axonal loss both within the LCST and the anterior corticospinal tract (ACST), raising questions as to whether axonal loss follows an initial demyelinating event. Both corticospinal tracts showed an increase in NRG1 protein, however, with a paradoxical decrease in both type I and III NRG1 mRNA expression. These same regions showed both increased microglial activation as well as sustained NRG1 receptor activation.

Conclusions: Corticospinal tract degeneration in sporadic ALS is associated with aberrant NRG1 expression and signaling, and microglial activation. The observation that NRG1 protein is increased in the absence of increased NRG1 mRNA expression, suggests that enhanced NRG1 signaling comes from descending central motor axons.

Discussion: Activation of microglia by NRG1 from central motor axons could explain the upper motor neuron signs seen in patients with sporadic ALS and may also be a therapeutic target to block disease progression.

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C7 NEUROPATHOLOGICAL FINDINGS OF SIX SCANDINAVIAN PATIENTS HOMOZYGOUS FOR THE D90A SOD1 MUTATION

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Keywords: SOD1, D90A, frontal lobe dementia

Background: The most common cause of familial amyotrophic lateral sclerosis (ALS) is mutations in superoxide dismutase-1 (SOD1). Currently more than 150 mutations in the SOD1 gene are known and one of the most frequent mutations is the change from aspartate to alanine in position 90 of the gene (D90A). This mutation is unique since it exists in both an autosomal dominant and a recessive form. In the Scandinavian pedigrees, it is inherited in a recessive manner and gives rise to a unique phenotype characterized by a predictive clinical pattern and long disease progression (1). So far, little is known about the neuropathological features of this specific mutation.

Objectives: To characterize and systematically investigate morphological findings in six autopsies of ALS patients homozygous for the D90A mutation in the SOD1 gene.

Methods: Brain and spinal cord sections from six autopsies were investigated by immunohistochemistry. Four different anti-peptide antibodies with specificity for misfolded/disordered SOD1 were used as well as antibodies directed at GFAP, ubiquitin, TDP-43, NF and cystatin C.

Results: All six patients showed a profound loss of motoneurons in the spinal cord and brainstem. In the remaining motonerons, numerous aggregates immunoreactive of misfolded SOD1 were found in the cytoplasm and occasionally also in the nucleus. Glial cells showed intranuclear reactivity for SOD1 in all patients but varied in number of glial cells involved.

Degeneration of the corticospinal tract and dorsal column was seen, as well as gliosis in the cortical areas of the frontal and temporal lobes and in the insula. Interestingly, these areas all showed microvacuolar degeneration of the superficial lamina, especially in layer II and III.

Discussion: Positron emission tomography (PET) studies on D90A patients using [^{11}C]flumazenil binding has revealed changes both in motor areas as well as in non-motor areas such as the left fronto-temporal and anterior cingulated cortices (2). Our present finding of pathological microvacuolar degeneration in the superficial layers of temporal and frontal cortices supports this notion.

Biochemcally, there are no significant differences in SOD1 activity between the D90A enzyme and the wild-type SOD1 enzyme (3). The aggregates of misfolded SOD1 that we find in the cytoplasm of motoneurons in D90A patients are morphologically the same type that can be found in the sporadic form of the disease.

Conclusion: Pathological changes in patients with the D90A SOD 1 mutation can be detected not only in motor areas of the central nervous system but also in different non-motor cortical areas. These findings indicate a possible frontotemporal lobar dementia in addition to motor neuron disease in these patients.

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C8 PROMINENT RE-DISTRIBUTION OF TDP-43 AND FUS/TLS UNDER CONDITIONS OF CELLULAR INSULT IN PRIMARY NEURONS

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Keywords: TDP-43, FUS, stress granule

Background: TDP-43 and FUS/TLS are nucleic acid binding proteins found to be mutated in sporadic and familial forms of ALS. Both proteins are implicated in multiple aspects of nucleic acid metabolism, including RNA splicing and transcription. In response to cellular stress, TDP-43 and FUS/TLS can relocate from the nucleus to the cytoplasm and accumulate in stress granules (SGs), which are ribonucleoprotein complexes where protein synthesis is temporarily arrested. It is not yet fully clear how TDP-43 and FUS/TLS proteins are redistributed from the nucleus to the cytoplasm under various cellular stresses and what is the significance of altered subcellular localization of these proteins in primary neurons.

Objectives: To investigate sub-cellular localization and accumulation of endogenous TDP-43 and FUS/TLS in primary spinal cord and cortical neurons exposed to oxidative and mitochondrial stress.

Methods: Primary neuronal cultures were harvested from mouse spinal cord and cerebral cortex. Neuronal cultures were treated with mitochondrial inhibitor; paraquat and oxidative stress inducer; sodium arsenite. Neuronal viability following treatments was assessed by MTT and LDH release assays. TDP-43, FUS/TLS and the SG marker TIAR were visualized by immunofluorescent staining.

Results: Neuronal viability is compromised in a time and concentration dependent manner in response to oxidative and mitochondrial stress. When treated at sub-lethal doses used to mimic stress conditions *in vivo*, TDP-43 and FUS/TLS re-distribute from the nucleus to the cytoplasm. In particular, treating spinal cord neurons with sodium arsenite causes changes to the subcellular distribution of both TDP-43 and FUS/TLS. In addition to formation of cytosolic aggregates resembling SGs, punctate, condensed staining of TDP-43 also appears in the nucleus of sodium arsenite treated spinal cord neurons.

Discussion and conclusions: Oxidative and mitochondrial stress affect viability and induce changes to TDP-43 and TLS/FUS sub-cellular localization in primary neurons. The cytosolic accumulation of TDP-43 and FUS/TLS induced by cellular stress demonstrates that oxidative or mitochondrial stress *in vivo* may induce aggregation of these proteins as a precursor to neuronal degeneration. We are now investigating the processes involved in cytosolic accumulation of the proteins. To our knowledge this study is the first to have reported aggregation of endogenous TDP-43 in the nucleus of spinal

cord neurons exposed to an oxidative stress-inducing agent, sodium arsenite. Further studies will be required to delineate the nature of the nuclear aggregates of TDP-43 and assess their significance in the pathology of ALS.

C9 DEVELOPING A TRANSGENIC ZEBRAFISH MODEL FOR TARDBP MUTATIONS IN ALS

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Keywords: zebrafish, TARDBP, development

Background: Mutations of the gene *TARDBP*, coding for the RNA binding protein TDP-43, have been identified in ALS patients, but the molecular mechanisms that cause the disease still remain uncovered. To assess the biopathology and develop therapeutic approaches of ALS, we use the zebrafish as a genetic model. We have previously functionally characterized a number of ALS-related *TARDBP* mutations by transient transgenesis in zebrafish for motor deficits (1).

Objectives: To develop a stable transgenic line overexpressing either mutant or WT *TARDBP* gene in zebrafish embryos and assess how this could cause or contribute to motoneuron degeneration linked to ALS. These lines will be useful for both understanding molecular mechanisms of disease and identification of modifiers of *TARDBP* functions as well as to screen small molecules that could eventually be used for the development of new ALS therapeutics.

Methods: We have generated two stable, inducible transgenic zebrafish lines expressing either the wild-type human *TARDBP* gene or the ALS-related mutation G348C. Our genes of interest were myc-tagged and placed under the control of the heat-shock inducible Hsp70 promoter to control the time of the expression and its level.

Results: In the absence of heat-shock, no myc-tagged protein expression was detectable. Heat-shocking embryos at 19 hours post-fertilization at 38.5°C for 30 min was sufficient to induce ubiquitous expression of TDP-43 in both lines, as assayed by RT-PCR, anti-myc immunoblotting and immunolabelling. Western blot analysis revealed an appropriate 50kD band for the myc-tagged protein in both lines and in addition revealed lower molecular weight bands only in the mutant line, presumably due to cleavage or degradation. As shown previously by immunolabelling in embryos transiently expressing TDP-43(1), stably induced mutant embryos showed significantly shorter and hyperbranched motor neuron axons compared to the control embryos. Moreover, the mutant embryos responded to touch stimuli but unlike WT embryos, the majority was unable to swim away, displaying weak and uncoordinated muscular contractions. Other neural cell types have been examined, and molecular partners of TDP-43 have been determined. In a preliminary screen of compounds used to treat neurological disorders, we have tested these for their effectiveness in treating the motor behavior and have identified several compounds which partially rescue the mutant phenotype.

Discussion and conclusions: These results indicate that zebrafish expressing a human *TARDBP* mutation related to ALS is a promising model for understanding ALS biopathology and advancing therapeutic drug discovery. The perturbation of motor neuron outgrowth may be an early indicator of mutant TDP-induced morphological anomalies causing functional disruption. These transgenic lines will be used for an in depth *in vivo* proteomic and genomic analysis and for a larger screen of small compounds.

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SESSION 2B HOLISTIC CARE AND QUALITY OF LIFE

C10 THE ROLE OF PALLIATIVE CARE IN THE MANAGEMENT OF ALS/MND

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ALS/MND is, from diagnosis, an incurable disease. As such it is a quintessential disease requiring the early involvement of palliative care. A systematic approach to the unfolding nature of the disease, careful planning ahead, meticulous symptom management, a rapid response to complications and clear communication to the patient, family and other health professionals are essential. The benefits of a multi-disciplinary approach to the illness and the importance of good end-of-life care shall be emphasised.

C11 ADDRESSING ISSUES OF SEXUALITY IN TERMINAL DISEASE

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Keywords: sexuality, relationships, palliative care

Background: There is a paucity of research that considers the experiences of people with a life-limiting illness in relation to sexuality and intimacy. Research that has explored the meaning of sexuality for this population has tended to focus on cancer (1), and the views of partners have not been identified (2). Apart from studies on sexual function (3), little is known about the impact of MND on sexuality.

Objectives: This qualitative study explores the lived experiences of sexuality and intimacy for people whose life is limited, and their partners.

Method: A Heideggerian, hermeneutic, phenomenological methodology was chosen, and a purposive sampling strategy was employed to recruit 27 'patients' and 14 partners of 'patients' living with a life-limiting illness (either terminal cancer or MND). It was not a requirement of the study that people were in a partnered relationship. One-to-one conversational interviews were audio-recorded and transcribed verbatim. Diekelmann and Ironside's (4) seven-stage hermeneutic process was used to analyse the narratives in order to uncover shared meanings.

Discussion of findings: Sexuality is an important aspect of peoples' lives that means different things to different people. When illness, disease or disability affects peoples' intimate and sexual relationships, some people are able to adapt and change, whilst others experience significant loss. When life is limited, this presents additional challenges.

This presentation begins with an overview of current research on sexuality and intimacy in palliative care. More detail is then given to illustrate the findings of this study as they relate to people with MND and their partners. For people living with MND, their coupled relationship is affected by a range of factors including bodily changes, disability, equipment that is intended to enable, and impending death. This research has shown that couples experience connecting and disconnecting as they move toward death. Where meaning is shared by both partners, re-connecting is possible. However, not all couples achieve this.

Conclusion: There is more to sexuality than erectile function. Telling people that their sexuality will not be affected does not recognise the psychosocial effects of MND or the practical and emotional interplay that is involved in sexual relationships. There is a role for health and social care professionals in supporting people to manage the changes that occur.

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C12 THE ROLE OF PSYCHOSOCIAL PHENOMENA IN THE DETERMINATION OF QUALITY OF LIFE FOR PATIENTS WITH MOTOR NEURONE DISEASE

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Keywords: quality of life, depression, coping

Background: Quality of life (QoL) for patients with MND has been commonly found to be unrelated to functional impairment. Whilst some research has been undertaken to evaluate factors that impact upon patient quality of life, to date no research has evaluated the direct and indirect causal links between psychological factors in a structured model for MND.

Methods: One hundred and seven patients with MND completed a suite of six questionnaires containing measures for

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fatigue, depression, anxiety, coping, functional status, social withdrawal and quality of life. The fatigue, depression, anxiety, coping and social withdrawal scales were modified in order to satisfy the demands of the Rasch model on a separate sample of 298 patients with MND. A hypothesised causal relationship between the study variables was tested using structural equation modeling (SEM).

Results: The final model was shown to have excellent fit characteristics ($\chi^2(5) = 6.06$, $p = 0.30$; $CFI = 0.99$; $GFI = 0.98$; $RMSEA = 0.045$). Quality of life (QoL) was primarily driven by strong direct effects from depression ($\beta = -0.47$, $p < 0.001$) and social withdrawal ($\beta = -0.34$, $p < 0.001$) in addition to an indirect effect of coping ability ($\beta = 0.35$, $p < 0.001$), mediated though anxiety ($\beta = -0.39$, $p < 0.001$) and depression ($\beta = -0.36$, $p < 0.001$). Fatigue did not impact directly upon QoL but exerted strong direct effects on anxiety, depression and social withdrawal ($p < 0.001$). Within this cohort, 7.50% of patients met the HADS criteria for probable depression and 13.10% for probable anxiety.

Discussion: This study highlights the importance of depressive symptomology and social withdrawal in the determination of patient QoL in MND. Coping ability was found to be a strong modifier of both depression and anxiety, and had a strong indirect effect upon QoL. Fatigue was shown to impact strongly upon anxiety, depression and social withdrawal although it did not elicit a significant direct effect upon QoL in this model.

Depression and anxiety are key factors in the determination of patient QoL, even when the majority of patients are not classified as clinically depressed or anxious. Also of key importance to the psychological health and quality of life for people living with MND is the adoption of coping strategies. The results show that patients who have difficulty coping are likely to experience higher levels of depression and anxiety, and resultantly experience poorer QoL.

If quality of life is to be maximised, it is crucial that patients who may have difficulty coping are identified and provided with adequate support and that symptoms of depression are closely monitored and treated accordingly. These results suggest that patients with MND may benefit from psychological counselling or group therapy in addition to their standard care.

C13 SOCIAL SUPPORT AND HEALTH-RELATED QUALITY OF LIFE OF ALS PATIENTS AND THEIR CAREGIVERS

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Keywords: health related quality of life, caregiver, caregiver load

Background: In ALS the social and psychological strains on patients and caregivers are often profound. However, it is unclear whether social and economic factors independently determine burden and quality of life of both patients and caregivers.

Objectives: To test the validity of a three-dimensional model integrating Health related quality of life (HRQoL) and the self-estimated social support with disease severity of ALS patients and their caregivers in Thuringia; and to identify independent predictors of these three aspects of ALS disease course.

Methods: We analyzed responses of patient-caregiver couples, house calls and self-help groups. To describe disease severity we used the ALSFRS-R and the Barthel Index. The EQ5D (HRQoL), BDI, SF 36 and F Sozu K14 scales were obtained from both patients and caregivers to measure both quality of life and degree of social support. To exclude manifest frontotemporal dementia, patients completed the FAB and the MMST. Caregiver burden was defined as the inverse of the ALSFRS-R of the corresponding patient as the 'caregiver load'. In every couple we collected distinguishing data about the patient and caregiver relationship, the amount of personal funds spent on the disease and the living conditions determined by available living space per occupant.

Results: 24 patient- caregiver couples completed the study. The particular results for the scales are ALSFRS-R (average $28.0 \pm SD9.5$) and 'caregiver load' (20.0 ± 9.5). The other scores for the patients/ caregivers were: EQ5D Index (25.5 ± 26.4)/(79.0 ± 17.3), F Sozu K14 (4.3 ± 0.6)/ (3.9 ± 0.9) and BDI (16.4 ± 9.3)/ (9.9 ± 6.7). The values of the ALSFRS-R are distributed significantly among all items of the EQ5D except pain/ discomfort in the patients. However, the 'caregiver load' showed no significant distribution in any item of the EQ5D. A univariate multiple analyses identified social support and severity of illness as significant impact factors on the HRQoL in ALS patients.

Discussion and conclusions: There are a number of impact factors on HRQoL in patients and caregivers who suffer from the multisystem disease ALS. We identified social support and severity of illness as two of them. The items of the EQ5D may be too unspecific to prove our definition of 'caregiver load' as a valid variable to recognize the burden of the caregivers. The separation of the 'caregiver load' in subgroups of the ALSFRS-R items may be a possible solution; however, it is more likely that caregiver burden must be derived from all three dimensions of our model. Further studies are required to identify independent components of these three dimensions with regards to perceived ALS disease course. Caregivers should be considered as patients' second order.

C14 PATIENT DECISION-MAKING IN MOTOR NEURONE DISEASE: THE VIEWS OF HEALTH PROFESSIONALS

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Keywords: patient decision-making, multidisciplinary care, barriers and enablers

Background: Multidisciplinary management of Motor Neurone Disease (MND) requires patients to make numerous decisions for their healthcare throughout the duration of the disease. However, the rapid progress of physical and cognitive symptoms present challenges to timely decision-

making (1). While these challenges are well known, there has been little investigation of the way MND clinical teams engage patients in decision-making for their care. Gaining such knowledge can promote stronger patient involvement in decision-making.

Objectives: The aim of the study was to explore the enablers and barriers to patient decision-making in MND care, from the perspectives of health professionals.

Methods: A focus group was conducted, using a semi-structured schedule to explore health professionals' experiences in patient decision-making. The issues discussed were derived from an in-depth review of the literature and opinion from experts in the field. Seven health professionals were recruited from a multidisciplinary, inter-service team specialising in MND care. The team was comprised of medical and allied health staff from three health services: palliative care; community-based rehabilitation and the Motor Neurone Disease Association of NSW (MNDA NSW). Transcripts were thematically analysed.

Results: Proactive partnerships between the patient, carer and the multidisciplinary team were thought to provide an environment for optimal and timely decision-making. However, decision-making remains hampered by the limited number of evidence-based treatment choices, and a lack of MND-specific resources for decision support. Five barrier groups to patient decision-making were identified: MND disease characteristics; limited evidence-based treatment options; quality and timing of information provision; access to multidisciplinary services; and the patient response to the diagnosis. Decision-making was seen to be enabled by prompt referral to an MND specialist clinic, early provision of MND-specific information, and early discussion of future care between the team, patient and carer. There was agreement on all barrier and enabler themes across palliative care, rehabilitation and MNDA staff groups.

Discussion and conclusions: Health professionals experienced in MND multidisciplinary care were able to identify specific enablers and barriers to patient decision-making. Reducing the barriers and identifying the enablers promotes improvement in the timing and quality of decisions. This exploratory study provides new empirical evidence for both generalist clinical teams and specialist MND services to better engage MND patients in healthcare decisions. The findings need to be compared with the perceptions of patients and their carers on the decision-making process.

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C15 DO HEALTH PROFESSIONALS CARING FOR PEOPLE LIVING WITH MOTOR NEURON DISEASE RECOGNISE THEIR OWN COMPASSION FATIGUE?

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Keywords: compassion fatigue, compassion satisfaction, burnout, health care professionals

Background: The diagnosis of ALS is unequalled in its devastating impact. As well as drastically shortening life of the sufferer, the physical and emotional damage that ensues, ripples outwards to involve family and friends. This aspect of ALS may partially explain why healthcare professionals can become highly emotionally involved with their patients. Whilst the majority find this a rewarding aspect of their work, one concern is that professionals may sometimes find it hard to compartmentalise these complex emotions in the context of the inevitable challenges that arise in their own personal lives outside work. Lack of awareness of a growing inability to cope can be potentially very harmful to personal and professional relationships. This study explores the concept of 'compassion fatigue' and 'burnout' and asks if healthcare professionals are able to recognise it in themselves, and if so, how they might try to manage it.

Method: A self reporting questionnaire (ProQOL), which assesses symptoms of compassion fatigue and professional quality of life, was distributed to 62 health care professionals caring for ALS patients using an online tool (www.surveymonkey.com).

Results: There was an overall response rate of 73%, (doctors 76%, nurses 62%, Care Centre Coordinators 93% and Regional Care and Development Advisors 68%). No individual from any professional group recorded a high score in measures of compassion fatigue or 'burnout', nor did they register a low score for compassion satisfaction. Health care professionals were good at identifying personal levels of compassion fatigue, burnout and compassion satisfaction. Over 90% of respondents said they valued informal support from a workplace colleague.

Conclusion: The results of this study would suggest that participants generally have a good understanding about their own levels of compassion fatigue, burnout and compassion satisfaction. This implies that an increase in awareness of the concepts of compassion fatigue and burnout might facilitate health care professionals in the accurate prediction of their own levels of compassion fatigue and enable them to take measures to evade it. Having a clearly defined support network among colleagues, and opportunities for formal debriefing after challenging cases are ways in which compassion fatigue can be avoided.

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SESSION 3A NEURODEGENERATIVE MECHANISMS: LESSONS FOR ALS/MND

C16 PRION DISEASES: A POTENTIAL LESSON FOR UNDERSTANDING THE PATHOGENESIS OF ALS

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Sequence-specific nucleated protein aggregation underlies the pathogenesis of most neurodegenerative diseases and constitutes the molecular basis of prion formation. Nevertheless, prion disorders have been distinguished from classical neurodegenerative diseases by virtue of their ability to be transmitted between individuals. In this lecture I will argue that prion-like propagation of pathogenic aggregated forms can explain the well-documented stereotypical spread of disease pathology in neurodegenerative disorders such as Huntington's, Lou Gehrig's, Alzheimer's and Parkinson's diseases. I will present data demonstrating that fibrillar polyglutamine aggregates like those associated with Huntington's disease can be internalized by mammalian cells in culture where they gain access to the cytosolic compartment and become co-sequestered in aggresomes together with components of the ubiquitin-proteasome system and cytoplasmic chaperones. I will also present recent unpublished data examining the biochemical and biophysical properties of protein aggregates and cell membranes that are necessary for cytoplasmic intrusion of aggregates and the implications for the pathogenesis and management of this class of conformational disease.

C17 MOLECULAR PATHOGENESIS AND TRANSLATIONAL RESEARCH IN SPINAL AND BULBAR MUSCULAR ATROPHY (SBMA)

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Keywords: SBMA, polyglutamine, androgen receptor

Spinal and bulbar muscular atrophy (SBMA) is an adult-onset lower motor neuron disease characterized by slowly progressive muscle weakness and atrophy. The cause of this disease is the expansion of a trinucleotide CAG repeat, which encodes the polyglutamine tract, within the first exon of the androgen receptor (AR) gene. Expanded polyglutamine tracts have been found to cause several neurodegenerative diseases including SBMA, Huntington's disease, several forms of spinocerebellar ataxia, and dentatorubral-pallidoluysian atrophy. In these disorders, known as polyglutamine diseases, the CAG repeat has a strong tendency to further expand, accelerating the disease onset with in successive generations.

SBMA exclusively occurs in adult males, whereas both heterozygous and homozygous females are usually asymptomatic. As for a transgenic mouse model of SBMA expressing the full-length human AR containing 97 CAGs (AR-97Q), neuromuscular symptoms are markedly pronounced and accelerated in the male mice, but either not observed or far less severe in the female counterparts. Androgen deprivation through surgical castration substantially improved the symptoms, histopathological findings, and nuclear accumulation of the pathogenic AR in the male AR-97Q mice. In contrast, subcutaneous injection of testosterone causes significant aggravation of symptoms, histopathological features, and nuclear localization of the pathogenic AR in the female AR-97Q mice. Since the nuclear translocation of AR is ligand-dependent, testosterone appears to show toxic effects by accelerating nuclear translocation of the pathogenic AR. Lending support to the ligand-dependent hypothesis are the clinical observations that manifestation of symptoms is minimal even in the females homozygous for an expanded CAG repeat in the AR gene, and that testosterone administration exacerbates neuromuscular symptoms in a patient with SBMA. In a large-scale randomized-controlled trial of clinical trial, leuprorelin treatment was associated with a greater reduction in barium residue than was placebo in patients with a disease duration less than 10 years difference between groups.

The ligand-dependent accumulation of the pathogenic AR, an initial step in the neurodegenerative process in SBMA, is followed by several downstream molecular events such as transcriptional dysregulation, axonal transport disruption, and mitochondrial insufficiency, indicating that both upstream and downstream molecular events should be targeted to therapy development. Although the precise mechanism by which motor neurons die remains unclear, activation of cellular defence reactions, ubiquitin-proteasome system, autophagy and heat shock proteins, has been shown to alleviate disease progression in animal models of SBMA. Restoration of transcriptional activity through histone acetylation is also capable of suppressing neurodegeneration in SBMA mice. Development of combination therapies will be the key for the translational research in SBMA.

C18 MECHANISMS OF PERIPHERAL AXONAL NEURODEGENERATION IN SPINAL MUSCULAR ATROPHY

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Keywords: spinal muscular atrophy, axon, excitability

Background: Spinal muscular atrophy (SMA) is a disorder of spinal motor neurons characterized clinically by the development of muscle weakness and atrophy. The 'up-front'

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clinical course suggests a substantial early loss of motor neurons followed by increasing stability of the surviving neurons with slow or no clinical deterioration and is unusual for a neurodegenerative disease. The mechanisms underlying this spectrum of differential survival and potential compensation of motor neurons in SMA remain unknown.

Objectives: To gain insights into axonal biophysical properties, disease pathogenesis and potential adaptations in SMA, the present study utilised clinical and functional assessments, combined with axonal excitability studies.

Methods: Axonal excitability studies were undertaken in 25 genetically characterized adolescent and adult SMA patients, stimulating the median motor nerve at the wrist. Multiple excitability indices (stimulus-response curve, strength-duration time constant, threshold electrotonus, current-threshold relationship and recovery cycle) were compared with 50 age-matched controls. Neurophysiological parameters were correlated with clinical and functional measures of disease severity, namely the MRC sum score and Spinal Muscular Atrophy Functional Rating Scale (SMAFRS) in SMA patients.

Results: In SMA patients there were reductions in CMAP amplitude ($P < 0.0005$) associated with reduction in stimulus response slope ($P < 0.0005$), confirming significant axonal loss. In the mild or ambulatory SMA patients, there was reduction of peak amplitude without alteration in axonal excitability; in contrast, in the non-ambulatory or severe SMA cohort prominent changes in axonal function were apparent. Specifically, there were steep changes in the early phase of hyperpolarisation in threshold electrotonus ($P < 0.0005$) that correlated with clinical severity. Additionally there were greater changes in depolarizing threshold electrotonus ($P < 0.0005$) and prolongation of the strength-duration time constant ($P = 0.001$). Mathematical modelling of the excitability changes in severe SMA patients supported a mixed pathology comprising features of axonal degeneration and regeneration.

Discussion and conclusions: The present study has established dysfunction of axonal K^+ and Na^+ conductances and alterations in passive membrane properties in SMA patients, supporting a mixed pathology of degeneration and regeneration. Importantly, excitability changes were most abnormal in the most clinically affected patients, critically indicating that the excitability changes relate to the process neurodegeneration and compensatory partial regeneration.

C19 ROLE OF SMN PROTEIN IN MOTOR NEURON DEGENERATION AND PROTECTION

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Keywords: survival motor neuron, spinal muscular atrophy, SOD1

Background: SMA results from insufficent levels of survival motor neuron (SMN) protein in spinal motor neurons and skeletal muscle. Reduced levels of SMN protein have been reported in mutant SOD1 models of ALS and recently spinal cords of sporadic ALS patients, in line with genetic association studies showing that low SMN levels may increase susceptibility to and/or severity of ALS. These findings raise the interesting prospect that SMN upregulation may be therapeutic in ALS which we tested here in transgenic ALS model mice.

Objectives: To investigate the effect of SMN upregulation on disease progression and neurodegeneration in transgenic $SOD1^{G93A}$ mice.

Methods: Transgenic $SOD1^{G93A}$ mice on either B6SJL or B6 congenic backgrounds were crossed with mice overexpressing human SMN driven by the prion protein promoter (PrP-SMN). Double transgenic $SOD1^{G93A}$ PrP-SMN mice and control genotypes $SOD1^{G93A}$ and PrP-SMN were examined for weight loss, motor function, disease progression and survival. Spinal cords were analysed by motor neuron counts, SMN immunohistochemistry and biochemical subcellular fractionation for SMN levels.

Results: Transgenic full-length SMN was overexpressed 2-fold in postnatal brains and spinal cords of PrP-SMN mice. In $SOD1^{G93A}$ PrP-SMN mice, human SMN overexpression was maintained up to 60 days, but depleted from 90 days, in accordance with endogenous Smn in $SOD1^{G93A}$ mice. SMN upregulation significantly delayed body weight decline, disease onset and preserved spinal motor neurons in $SOD1^{G93A}$ PrP-SMN animals, despite limited effects on symptom progression.Elevated SMN levels in cytoplasmic and nuclear fractions were confirmed in spinal cords of doubly transgenic mice.In contrast to PrP-SMN mice, SMN-positive gems were drastically reduced in motor neurons in $SOD1^{G93A}$ and $SOD1^{G93A}$ PrP-SMN animals.

Discussion and conclusions: These results suggest that SMN protein depletion in spinal motor neurons may be a determinant of neuronal vulnerability and loss in models of ALS. SMN upregulation using a transgenic approach protects against early phases of disease and neurodegeneration in mutant SOD1 mice. The mechanisms underlying protection of motor neurons by overexpressed SMN in this model is currently under investigation.

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SESSION 3B TRANSLATING EVIDENCE INTO PRACTICE

C20 TREATING MND: DOES THE EVIDENCE LEAD US OR LAG BEHIND US?

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Keywords: treatments, evidence-based, experience-based

The motor neuron diseases are a heterogeneous and complex spectrum of disorders which implicate a multidisciplinary approach to treatment. The spectrum of signs and symptoms contributing to disability are vast yet the number of established treatments which have been justified by outcomes data are not. Despite this, we continuously introduce novel treatment paradigms into our clinics, built upon our collective experience treating MND patients.

As academics we are oriented to turning our experience into evidence. As clinicians we are oriented to treating our experiences as if they were evidence. The chiasm between the two approaches can be significant and complicated given the phenotypic diversity and sense of urgency brought out in patients with MND.

This presentation will first highlight the justification for the existing standards underlying evidence based guidelines in the US and Europe. Furthermore a great variety of treatments which we apply selectively in routine practice will be surveyed and comments will be offered regarding their potential to ever be subjected to formal outcomes testing.

Our individual biases in routine practice are often shaped by experiences which may pertain to selective symptom combinations or specific disease presentations. These can be readily recognized but difficult to capture in a clinical trial. If evidence guidelines are accepted as the best 'standard of care', what role should we prescribe to often more efficacious and numerous non-evidence based interventions that we commonly adopt.

As we draw lines between evidence and experience we struggle with the essence of our purpose in treating MND patients and moving our field forward. The balance between the two may serve as the most significant driving force in identifying novel breakthroughs. Reliance on evidence alone can be as misdirected as a reliance solely on experience. The controversy raised by this dichotomy will be discussed and the perspective of the patient, practitioner and academic physician will be contrasted.

C21 DOES RIGOROUS CONTROL OF EXERCISE INTENSITY AFFECT SURVIVAL AND FUNCTIONAL OUTCOMES IN ALS?

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Keywords: exercise control, oxygen uptake, aerobic work efficiency

Introduction: The uncertainty about exercise in ALS is mainly derived from a paucity of studies, different approaches and lack of rigorous control of exercise intensity, which can be evaluated by gas exchange on cardio-pulmonary exercise stress test protocol (CPET). We aimed to test the efficacy of applying exercise treadmill ramp protocol leveled 20% lower than determined by CPET.

Methods: Prospective, randomized single-blinded controlled-trial. Forty consecutive ALS patients with no other medical condition and able to perform CPET regardless of their FVC, were assigned to group 1 (G1, n = 20) and group 2 (G2, n = 20) as determined by their residential area. Patients in G1 underwent a supervised-exercise program 3 times/week, with non-invasive ventilation (NIV) or Body Weight Supporting System if required. Patients in G2 exercised to fatigue (Borg scale) with no supervision (with or without NIV). All patients performed CPET at admission and during follow-up (3, 6, and 12 months). ALSFRS-R scores and respiratory function tests (RFT) were performed at 3 month-intervals. The main outcomes were: the minute ventilation in L/min^{-1} (VE); the peak oxygen uptake expressed in L/min^{-1} (VO^2 pk) or in metabolic equivalents (METs), the carbon dioxide output (VCO^2); the ratio oxygen uptake/work rate (VO^2/WR) as well as ALSFRS-R scores and their respective slopes. Survival analysis was a secondary outcome.

Results: No clinical or laboratory measurement was different between groups at baseline, except for the ratio VO^2 pk/WR that was greater in G1 ($p = 0.01$) that was not found 3 months later. At admission, VO^2 pk m ± sd (1.015 L/min ± 0.301; $p = 0.9$) were under the normal range of the predicted and did not change overtime. VE m ± sd (24.672 L/min ± 11.25; $p = 0.7$) was low but above the 50% of the predicted value and doubled the initial value in G1 ($p = 0.001$). The rate of decline of all ALSFRS-R scores at 3, 6 and 12 months was significantly slower for G1. Survival from symptoms onset to

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April 2011 was not different in the two groups but survival from rehabilitation onset was significantly different (longer for G1, 512 days, than for G2, 318 days, p = 0.04, Kaplan-Meyer estimates with Log-rank test).

Discussion: Deconditioning was not observed. Despite the reduced VO^2 pk at admission, no changes in the follow-up were identified. The high ratios VO^2 pk/WR reflected the initial aerobic work inefficiency with diminished breathing reserve. In G1 we observed a significant improvement in the VE and the WR, along with a reduced decline of functional scores. Survival after exercise onset was longer in G1. We conclude that a well-controlled exercise protocol as defined by CPET is beneficial to ALS patients even with low FVC values.

C22 IMPLEMENTATION OF AMERICAN ACADEMY OF NEUROLOGY (AAN) AMYOTROPHIC LATERAL SCLEROSIS (ALS) GUIDELINES AS PERFORMANCE MEASURES IN THE JOINT COMMISSION DISEASE SPECIFIC CERTIFICATION (DSC) PROGRAM AT CAROLINAS NEUROMUSCULAR/ALS-MDA CENTER

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Keywords: performance measures, TJC (The Joint Commission), DSC (Disease-Specific Certification)

Background: DSC has been designed to recognize disease management programs for superior quality. A forerunner was the Primary Stroke Center Certification developed in collaboration with the American Stroke Association which depends on a Stroke Core Measure Set and Stroke Performance Measure Requirements based on the Brain Attack Coalitions' guidelines. In the past, many ALS clinics participated in ALS CARE, a paper-based documentation of patient care encounters longitudinally that permitted assessment of each ALS Clinic's adherence to the 1999 AAN ALS Practice Parameter (AANALSPP) (1) and benchmarks for ALS Care provided in the real world (2,3). The ALS Association Center[SM] Program voluntarily certified ALS Centers based on the facilities and personnel available but did not require a longitudinal analysis of patient outcomes. The recently expanded 2009 AANALSPP (4) is the basis for developing ALS DSC in collaboration with the Muscular Dystrophy Association (MDA) and the AAN to improve patient safety and quality (5).

Objective: Implement and evaluate core performance measures of the ALS DSC profile based on 2009 AANALSPP and collect quantitative patient data for outcomes assessment in a large ALS clinic.

Methods: Standardized performance measures: 1) Mini-Mental Status Examination (MMSE); 2) Patient Health Questionnaire (PHQ-2); 3) Patient Health Questionnaire (PHQ-9); 4) Hendrich II Fall Risk Assessment (HIIFRA) and 5) Respiratory Management Assessment (RMA) measured patient status according to 2009 AANALSPP. Monthly and quarterly audits of performance were ascertained across 689 ± 175 (SD) annual ALS encounters through 2009-2011.

Results: Cognitive (93.3 ± 11.7%), psychiatric-screening (PHQ-2) (87.6 ± 21.0%), psychiatric-followup (PHQ-9) (84.4 ± 27.6%), falls (93.5 ± 9.9%) assessments were performed according to practice standards achieving benchmarks with wide confidence limits. Cognitive and psychiatric measures formed the basis of each patient's assessment at that encounter (98.5 ± 5.9%). Falls risk (HIIFRA) used initially to monitor falls was discordant from the number of falls experienced during the inter-clinic interval and was replaced with real-time assessment of falls between last and current clinic visit. Subsequent to this change, number of falls were recorded in each clinic note (89.5 ± 9.7%).Respiratory management assessments identified functional vital capacity (FVC) measurement was high but not universal (95.4 ± 7.2%).

Conclusion: Initiating five performance measures in an ALS Clinic requires increased encounter time and increased administrative time. In the first 6 months of implementing and evaluating performance measures in an ALS Clinic, we realized that a standard Falls Risk Assessment scale did not meet the needs of assessing falls risk in ALS patients and corrective measures were taken.

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C23 EVIDENCE-BASED GUIDELINES FOR POWER WHEELCHAIR PRESCRIPTION FOR PERSONS WITH ALS/MND

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Keywords: power wheelchair, guidelines, power features

Background: Recent publication by Ward, *et al*. in February 2010 (1) began the process towards developing essential components on a power wheelchair for persons with ALS/MND to meet their long term needs. One other article exists which also looks at power wheelchair (PWC) use in ALS/MND from 2001 (2). Rapid progression of the disease means the PWC must be flexible for long-term needs for comfort and function.

Objectives: To survey persons with ALS at 1 month, 6 months and a year after receiving PWC to develop guidelines for what is required for long term effective use, comfort and function of their PWC, and to use these surveys for evidenced-based guidelines.

Methods: 33-question survey and Psychosocial Scale of Assistive Devices sent at 1 month after getting a new PWC, follow up survey sent at 6 months and 1 year as well. The survey addressed satisfaction, feature use, comfort and function with the PWC.

Results: Based on satisfaction and feature use survey results, we are proposing requirements for what should be ordered on a new PWC for a patient with ALS/MND as well as features which would be helpful but are not required. At 1 month, 38% of users are using tilt, recline and elevating legs features at least 2 times a day, and at 6 months 55% are performing these tasks. For users at 1 month, 88% are still pleased with their choice of cushion, headrest and power features, and this percentage drops only slightly at 6 months.

Discussion: The proposed guidelines include that the PWC should have tilt, recline and power elevating foot platform as power features, upgraded electronics, power features run through the joystick, contoured backrest, pressure relieving and positioning cushion and plush headrest. Other features which are helpful but not required include a seat elevator, alternative drive controls, laterals and thigh guides for positioning support, supportive armrests, and switches for on/off and mode.

Conclusion: Our proposed evidence-based guidelines for PWC users with ALS/MND are currently open for comment and discussion. We feel our multidisciplinary ALS/MDA clinic has a responsibility to develop guidelines and help provide evidence for the long-term needs of these patients and their PWC because of the focused treatment and wheelchair evaluations, which are performed daily at our center.

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C24 A SYSTEMATIC REVIEW OF ALS SERVICE USERS' PERCEPTIONS OF SERVICES AND DECISION MAKING IN CARE

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Keywords: care preferences, decision making, health care services

Background: Effective symptom management, physical and psychological support, are components of ALS care. Service users and providers of palliative services can hold different perspectives on the benefits of care. However, few studies have explored the delivery of services from the ALS service user's perspective.

Objectives: To examine the literature on ALS service users' perceptions of health care services; to draw attention to factors that may influence preferences for care.

Method: A review of the literature from 1988 to March 2011 was undertaken. Databases used included; Medline, Cinahl, AMED, PsycInfo, Cochrane Library, Evidence Based Medicine Reviews, Science Citation Index, Social Sciences Citation Index, and Arts and Humanities Citation Index. Search terms used included; '*amyotrophic lateral sclerosis'* or *'motor neurone disease'* and/or '*services', 'healthcare', 'experiences', 'expectations', 'satisfaction', 'decision-making', 'perceptions', 'perspectives'* and *'care preferences'*. Separate manual searches of online editions of palliative care journals (including early online where available) were also undertaken using search terms '*motor neurone disease'* and '*amyotrophic lateral sclerosis'*. A narrative approach was used to synthesise studies (1).

Results: Studies of decision making and preferences for care have focussed primarily on end-of-life intervention. Only few studies report on service users' decision making in services prior to end-of-life care. According to existing literature, dissatisfaction with services relates to absence of specialised care; limited access to assistive devices; inadequate respite care and emotional support; delays in diagnosis; concerns regarding method of disclosure; and a lack of knowledge about ALS among professionals. Satisfaction with services is confined primarily to the use of assistive devices. Service users also seek autonomy and exert control when making decisions about care. The need to exert control remains stable overtime. However, care preferences change to accommodate to evolving perspectives and support systems.

Discussion: The literature suggests that ALS service users expect dignified care but they have unmet expectations of their care. A combination of personal values, desire to maintain control, perceptions about quality life, social and carer support determine service users' preferences for and decisions about care. Some service users may resign themselves to the inevitability of ALS. However, the majority seek a broad range of services and some maintain positive perceptions about health.

Conclusion: ALS service users make choices about care that are grounded in how they interpret their own lives and how they judge potential benefits of care. Further research on how service users interact with services and decide about care is recommended. Research on the delivery of services that are sensitive to service users' preferences for care is warranted.

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SESSION 4A RNA AND PROTEIN PROCESSING

C25 ALTERED RNA FUNCTION IN ALS: LESSONS FROM GENETICS

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Keywords: RNA, genes, mutations

Amyotrophic lateral sclerosis (ALS) is a degenerative disorder that selectively targets motor neurons in the brain, brainstem and spinal cord. At least 10% of cases are caused by gene defects that are transmitted as dominant traits. Multiple gene mutations have now been identified as causes of either ALS or ALS and frontotemporal dementia. These mutations highlight several themes in ALS pathogenesis. Studies of mutations in the SOD1 gene and protein have highlighted protein instability as an upstream factor in this disease. By contrast, other recently defined ALS genes, prominently including TDP-43 and FUS/TLS, identify perturbations of RNA function as fundamental components of motor neuron disease. Multiple aspects of RNA biology are potentially implicated including transcription, splicing, shuttling between the nucleus and cytoplasm, protein translation and activity-dependent local control of protein synthesis in dendritic spines. These defects, which may be particularly critical after cellular stress, entrain a complex series of downstream events (e.g. mitochondrial dysfunction, excitotoxicity, disturbances of axonal transport) that ultimately lead to the demise of the motor neuron, with relevance both to familial and sporadic ALS. This presentation will review these emerging concepts as well as implications for further investigations of the role of gene variations in ALS pathogenesis.

C26 IDENTIFICATION OF FUS/TLS-MEDIATED RNA-PROCESSING ALTERATIONS IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: FUS/TLS, RNA targets, CLIP-seq

Background: TDP-43 and FUS/TLS, two RNA/DNA binding proteins, are central in the pathogenesis of Amyotrophic Lateral Sclerosis (ALS), yet their physiological roles in the central nervous system are poorly understood. Using cross-linking immunoprecipitation coupled with high-throughput sequencing (CLIP-seq) we recently identified binding sites in 6,304 genes as the brain RNA targets for TDP-43. Following depletion of TDP-43 from mouse adult brain with antisense oligonucleotides, we found that levels of 601 mRNAs were changed and 965 splicing events were altered. RNA-targets whose levels were most depleted by reduction in TDP-43 were derived from genes that are essential for the maintenance of neuronal integrity. A fundamental remaining issue is the precise role(s) of FUS/TLS in RNA metabolism regulation and how alterations in its properties may underlie neurodegeneration. Like TDP-43, FUS/TLS has been proposed to participate in several steps of RNA processing, including alternative splicing and transcription regulation. Few RNA targets of FUS/TLS have been identified and a comprehensive protein-RNA interaction map still needs to be defined.

Objectives: To identify *in vivo* RNA targets of FUS/TLS, to validate the roles of FUS/TLS in the processing of these targets and to determine TDP-43-FUS/TLS overlapping mRNA targets and/or RNA-processing alterations.

Methods: We have used cross-linking immunoprecipitation CLIP-seq to identify RNAs bound by FUS/TLS in mouse brain. We have subsequently determined the effects of FUS/TLS loss of function on RNA expression and splicing patterns by using high-throughput sequencing of cDNA (RNA-seq) and splicing-sensitive arrays.

Results: Greater than 8 million uniquely mapped reads enabled the accurate generation of clusters using gene-specific thresholds to define FUS/TLS binding sites. To validate the role of FUS/TLS in the regulation of transcription and alternative splicing via these sites, downregulation of FUS/TLS *in vivo* was achieved in an otherwise normal adult mouse brain, using direct injection of antisense oligonucleotides against FUS/TLS. Transcriptome profiling from brains with FUS/TLS reduction to 90% of endogenous levels confirmed its roles in alternative splicing and gene expression regulation.

Discussion and conclusions: Genome-wide identification of validated RNA targets is a first step in the elucidation of the molecular mechanisms underlying death of motor neurons in ALS. Since mutations in either TDP-43 or FUS/TLS cause a similar disease phenotype, and both proteins are involved in the same RNA-processing steps, we anticipate that the RNA-targets affected by both TDP-43 and FUS/TLS may be the most relevant for disease. This study reinforces the crucial role of RNA-processing regulation for neuronal integrity and potentially identifies candidate genes whose altered processing is central to ALS pathogenesis.

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C27 PATHOLOGICAL FRAGMENT OF TDP-43 IN ALS IS GENERATED BY ALTERNATE TRANSLATION INITIATION

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Keywords: TDP-43, RNA, splicing

Background: TAR DNA binding Protein-43 (TDP-43), is a major component of the cytoplasmic inclusions characteristic of amyotrophic lateral sclerosis (ALS).In addition to the full-length protein of 43 kDa, biochemical profiles of TDP-43 from diseased tissues have shown the presence of lower molecular weight species, most predominantly of 35 kDa (TDP-35) and 25 kDa (TDP-25). These species have been considered caspase-3 (C-3) degradation products since cleavage at the C-3 consensus sequences DETD89A and DVMD219V can generate species of 35 kDa and 25 kDa *in vitro*, respectively. However, there is no direct proof that these species are generated by C-3 cleavage in disease.

Objectives: Since TDP-43 is a splicing regulator and is itself alternatively spliced, we explored the possibility that the lower molecular weight species of TDP-43 found in diseased tissues may not be C-3 degradation products, but instead, alternatively spliced (AS) variants of TDP-43.

Methods: Genome bioinformatics databases were mined for AS transcripts of TDP-43. We focused on one AS transcript that showed elevated expression in ALS spinal cord compared to controls, as assessed by RT-PCR.This transcript was cloned and expression studies undertaken in cultured SHSY5Y human neuroblastoma cells and primary motor neurons. Antibody specific to the splice variant was generated and used to assess its expression in ALS spinal cord tissues.

Results: We identified an AS variant of TDP-43 with 91 bp skipped in exon 2 that was upregulated in ALS spinal cord compared to controls. The 91bp deletion caused a frameshift and use of downstream alternate translation initiation codon, ATG ^{Met85}. Expression of this transcript in cells generated a species of 35 kDa, named AS-TDP-35, that partitioned to the urea soluble fraction, and formed cytoplasmic aggregates both in SHSY5Y cells and primary neurons, causing neurotoxicity. To determine if TDP-35 is generated by expression from Met85 (AS-TDP-35) or C-3 cleavage at DEVD89A (C3-TDP-35), we made neoepitope antibodies corresponding to the different N-terminal sequences that would be generated, respectively. We show that the pathological TDP-35 species observed on immunoblots of ALS lumbar spinal cord tissues is labeled with antibody specific to AS-TDP-35, but not C3-TDP-35 antibody. We also show that AS-TDP-35 antibody labels both skein-like and round inclusions, whereas C3-TDP-35 antibody labeling was negative.

Discussion: These results show that the lower molecular weight TDP-43 species of 35kDa present in ALS spinal cord tissues is generated by expression from Met85. We propose that this occurs through expression of an AS variant of TDP-43, in which there is a 91 bp deletion in exon 2, causing a frameshift and alternate translation initiation. This identifies abnormal splicing of TDP-43 to generate TDP-35 as well as the downstream neurotoxic effects of its expression, as potential therapeutic targets.

C28 INTRACELLULAR INCLUSIONS OF THE RNA BINDING PROTEIN RBM45 IN ALS AND FTLD PATIENTS

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Keywords: RNA binding protein, protein aggregation, intracellular inclusion

Background: The emergence of defects associated with RNA binding proteins, predominantly TDP-43 and FUS, as pathogenic mechanisms in ALS and frontotemporal lobar dementia (FTLD) has fundamentally changed our view of these diseases. These proteins form cytotoxic inclusions in motor neurons and often exhibit an aberrant cytoplasmic localization. Moreover, genomic studies have identified sequence alterations in the TDP-43 and FUS genes in familial forms of ALS. We recently used unbiased proteomics methodologies to identify proteins that exhibit abnormal levels in the CSF of ALS patients. We detected an increase in the novel RNA binding protein RBM45 in the CSF of ALS patients.

Objectives: We used a combination of immunohistochemistry, immunofluorescence microscopy, and immunoblot to characterize RBM45 protein expression and distribution in control, ALS and FTLD patients.

Methods: Paraffin tissue and snap frozen tissue from the spinal cord, motor cortex and hippocampus of ALS, FTLD, and age-matched control patients were obtained from the University of Pittsburgh ALS Tissue Bank. RBM45 expression and subcellular distribution were examined by immunohistochemistry, immunofluorescence, and immunoblot. We also used anti-TDP43 to examine co-localization with RBM45.

Results: Immunoblot analysis verified our mass spectrometry results and demonstrated increased RBM45 levels in the CSF and spinal cord of ALS patients. We observed weak RBM45 immunoreactivity in the nucleus of motor neurons and glial cells in the spinal cord and brain in control subjects. However in ALS patients we detected robust RBM45 nuclear immunostaining and intracytoplasmic inclusions of RBM45 in spinal cord motor neurons and dendate granule cells of the hippocampus. We also observed cytoplasmic inclusions of RBM45 in the dendate gyrus of FTLD patients. RBM45 inclusions bear a striking resemblance to those containing TDP-43 or FUS in ALS/FTLD. In some cases, RBM45 inclusions co-localized within TDP-43 inclusions. However many RBM45 inclusions did not contain TDP-43. No inclusions were observed in control subjects. We also identified prominent glial RBM45 immunoreactivity in all brain areas of interest.

Discussion: We have identified a new RNA binding protein that exhibits intracellular inclusions of affected neurons in ALS and FTLD. Our results support the role for RNA binding proteins in the pathogenesis of ALS and FTLD. The overlapping distribution of RBM45 and TDP-43 suggests a commonality between the mechanisms that result in inclusion formation for each protein. Our findings raise the possibility that RBM45 expression can be induced in response to pathologic stressors. Moreover, we found prominent glial expression of RBM45.

Conclusions: We identified intracellular inclusions of the novel RNA binding protein RBM45 in affected neurons of ALS and FTLD patients. Ongoing studies will determine the functional role of RBM45 in regulating mRNA metabolism and its role in ALS and other neurodegenerative diseases.

C29 DOWNREGULATION OF RNA EDITING ENZYME ADAR2 AND SPORADIC ALS

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Keywords: glutamate receptor, neuronal cell death, RNA editing

Introduction: Ca^{2+}-permeable AMPA receptors play a pivotal role in neuronal death in ALS. GluA2 is a subunit of the AMPA receptor, playing a key role in the regulation of Ca^{2+} permeability after adenosine to inosine conversion (RNA editing) at the glutamine/arginine (Q/R) site, where the Q codon (CAG) is substituted by the R codon (CIG = CGG). A subset of motor neurons of patients with sporadic ALS express unedited (Q at the Q/R site) GluA2, hence abundant Ca^{2+}-permeable AMPA receptors. Because GluA2 Q/R site-editing is specifically mediated by adenosine deaminase acting on RNA 2 (ADAR2), it is likely that ADAR2 activity is reduced in ALS motor neurons.

Methods: Using a laser-microdissector, single motor neurons were dissected from autopsy-obtained frozen spinal cords of 29 ALS patients. After analyzing the extent of GluA2 Q/R site-editing in an individual motor neuron, expression levels of three members of the ADAR family (ADAR1, ADAR2 and ADAR3) were analyzed on pooled cDNAs from ALS motor neurons expressing unedited GluA2 and those expressing only edited GluR2. In addition, enzymatic activities of ADAR1 and ADAR2 in ALS motor neurons were analyzed by the measurement of the extent of RNA editing positions specifically mediated by either one of ADARs. The results were compared with those on control subjects and patients with other neurological diseases.

Results: We demonstrated that a considerable proportion of motor neurons express unedited GluA2 in all the ALS cases examined, while all the motor neurons of control cases expressed only edited GluA2. ADAR2, but not ADAR1 or ADAR3, was significantly downregulated in all the motor neurons of ALS patients, more extensively in those expressing unedited GluA2 than those expressing only edited GluA2. Extents of RNA editing at ADAR2-specific RNA editing positions, but not those at ADAR1-specific RNA editing positions were significantly lower in ALS motor neurons than in control motor neurons.

Discussion: The present results demonstrate that ADAR2 is universally downregulated in motor neurons of sporadic ALS patients irrespective of the variety of clinical manifestations. This indicates that loss of ADAR2-immunoreactivity in about half of motor neurons of ALS patients (1) is not likely a consequence of accelerated degradation of ADAR2 proteins but reflects the severity of ADAR2 downregulation. Because loss of ADAR2 induces death of motor neurons specifically by failure to edit the GluA2 Q/R site in conditional *ADAR2* knockout mice (2), our results suggest that progressive downregulation of ADAR2 is involved in the ALS pathogenesis.

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SESSION 4B COGNITIVE CHANGE

C30 THE CLINICAL SPECTRUM OF COGNITIVE CHANGES IN MND

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There is growing evidence for an overlap between MND and Frontotemporal Dementias (FTD) at both a clinical and pathological level. About 10% of patients with FTD develop clinical MND but a much higher proportion have more subtle evidence of motor tract dysfunction. Viewed from a MND/ALS clinic perspective, a significant proportion of people with MND manifest features of FTD (either changes in behaviour/social cognition, higher order cognitive abilities such as planning, organizing and motivation or in language abilities). This talk will concentrate on the later group and outline the range of FTD features that can occur in MND as well as methods of detection and measurement using modern neuropsychological tests. I will draw on the results from a NSW wide survey of 100 patients and caregivers that was recently conducted confirming that there is indeed a high rate of FTD like features in MND, notably apathy, which have a significant impact on caregiver burden. I will also describe a study of cognition and behaviour in consecutive patients attending a MND clinic in Sydney confirming that about a quarter of MND patients fulfill criteria for FTD while a higher proportion have some behavioural and/or cognitive features. The most sensitive task appears to be one directed at inhibitory control that reflects dysfunction of orbitofrontal cortex. These findings clearly have implications for patient management.

C31 MOTOR NEURON DISEASE AND FRONTOTEMPORAL DEMENTIA: THE ROLE OF LANGUAGE DYSFUNCTION

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Keywords: language, dementia, aphasia

Background: From the very beginning of the scientific description of MND, clinicians identified two forms of cognitive impairment: a full-blown dementia, reminiscent of Frontotemporal Dementia (FTD), and subtle cognitive changes observed in non-demented MND patients (1). Supported by recent discoveries in molecular biology, linking together the pathologies of MND and FTD, cognitive and behavioural changes are now recognised as an important feature of MND (2).

However, several questions remain open (1). Firstly, it is not clear, whether MND/Dementia forms a separate entity, distinguishable from the "classical MND", or constitutes an end of a spectrum of cognitive changes in MND. Secondly, while it has been recognised that cognitive and behavioural features can occur independently in MND patients (2), the role of language impairment in this context is less clearly defined (3). Finally, questions remain about the relation between MND and FTD. FTD can present in three forms: behavioural variant FTD (bvFTD), Non-fluent progressive aphasia (NFPA) and Semantic Dementia (SD). It has not yet been determined whether MND is associated with a combination of the features of all three, or whether MND/fvFTD, MND/NFPA and MND/SD can occur as separate clinical entities.

Objectives: 1) To compare the cognitive/behavioural profiles of MND/Dementia with non-demented MND patients, with particular emphasis on language dysfunction; 2) to discuss their similarities and differences with the three subtypes of FTD.

Methods: We compared clinical and cognitive data of 20 patients with MND/Dementia and 40 non-demented MND patients from two research centres (Cambridge and Edinburgh), against a background of a thorough literature review, going back to the early 20th Century.

Results: In the MND/Dementia group, all patients showed evidence of language dysfunction, usually taking the form of mutism, with a profound impairment of syntactic comprehension and a particular difficulty in processing verbs/actions. More subtle language impairment was found in the non-demented group. It manifested itself in spelling errors, mild deficits in syntactic comprehension and a semantic deficit characterised by a predominant impairment in understanding of sequential/syntagmatic as opposed to parallel/paradigmatic relationship between actions. Interestingly, this pattern was reversed in the only patient presenting with the clinical picture resembling SD. In both groups, the level of language impairment was not related to the severity of dysarthria, motor and cognitive/behavioural symptoms.

Discussion and conclusions: Language impairment is prominent in both MND/Dementia and in non-demented MND patients. It shares some features but is not identical to any of the three subtypes of FTD. We postulate that language impairment forms an independent clinical feature of MND. Language assessment is, therefore, of central importance to our understanding of MND/FTD.

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C32 EMOTION PROCESSING IN MND AND FTD

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Keywords: cognition, emotion, frontotemporal dementia

Background: Although initially considered a pure motor syndrome, recent evidence has shown that Motor Neurone Disease (MND) is a multisystem disease which can overlap with frontotemporal dementia (FTD) (1). While only 15% of patients with MND develop frank FTD, up to 50% of cases may exhibit some degree of cognitive impairment (2). Despite the fact that FTD patients experience prominent deficits in emotion perception and social cognition (3), these domains have received relatively little attention in MND, and no study has directly compared FTD and MND patients.

Objectives: The aim of the current study was to investigate basic emotion processing abilities in MND and FTD patients in order to: 1) determine the degree of impairment found in MND patients when processing emotional stimuli, and 2) compare the severity and nature of these impairments in MND and FTD patients when tested on the same measures.

Methods: Twenty-six patients with a diagnosis of MND (13 with co-existing FTD (FTD-MND) and 13 without dementia), 13 behavioural FTD patients and 28 healthy controls completed a battery of neuropsychological and emotion tasks. Cognitive domains included memory, language and executive functions. For the emotion tasks, participants were instructed to select the emotional label associated with black and white photographs of faces, or short video clips of actors portraying basic emotions.

Results: Both MND ($n = 26$) and FTD patient groups showed significant deficits on the emotion tasks compared to controls. However, after dividing MND patients into those with and without co-existing FTD, only the FTD-MND patients showed significant impairments on measures of emotion, whereas patients without frank dementia performed normally. FTD-MND and FTD patient groups displayed similar levels of impairment on emotion measures, even after controlling for measures of general cognition.

Discussion and conclusions: Patients with MND who also meet clinical criteria for FTD show significant deficits on tests of emotional processing, over and above deficits found on general tests of cognitive function. In this group, disturbance of emotion processing appears to be at least as severe as that seen in patients with FTD. By contrast, MND patients who do not show features of FTD appear to have preserved emotion processing, which clearly has important clinical implications. Specific profiles within the MND groups will be discussed.

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C33 A BRIEF COGNITIVE ASSESSMENT IN AMYOTROPHIC LATERAL SCLEROSIS AND ITS CORRELATION WITH DETAILED NEUROPSYCHOLOGICAL TESTING

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Keywords: FTLD, language, diagnosis

Background: Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are neurodegenerative disorders with a shared pathologic feature of neuronal lesions immunoreactive to TAR DNA binding protein of 43 kD (TDP-43). FTLD patients can present with clinical ALS, and ALS patients can develop behavior or language disorders resembling FTLD. ALS patients can also develop cognitive impairment with prominent executive or language deficits short of dementia, and it is thought that cognitive impairment in ALS is mediated through executive dysfunction. We hypothesized that cognitive impairment in ALS can be accurately detected using a brief cognitive assessment, and compared the diagnostic accuracy of this assessment with two other diagnostic measures of cognitive impairment.

Objectives: To determine the prevalence of cognitive dysfunction in a cross-sectional cohort of ALS patients in a multi-disciplinary ALS clinic in a university medical center setting, and to compare the diagnostic accuracy of a brief cognitive assessment based on the Philadelphia Brief Assessment of Cognition with the Frontal Behavioral Inventory (FBI) and detailed neuropsychological analysis.

Methods: Cognitive functions were assessed in 154 ALS patients using a 4-item brief cognitive assessment, including working memory (reverse digit span), letter-guided fluency, oral trails, and delayed verbal recall. The FBI was also administered to each patient's caregiver or study partner. 21 patients underwent detailed neuropsychological analysis to include assessments for verbal and visual learning and recall, along with executive, language, and visual spatial functions.

Results: Among 154 ALS patients (64% women, average age 60.2, S.D. 11.2 yrs) who underwent the brief cognitive assessment, 76 (49%) had impairment in at least one cognitive test (1.5 standard deviation below the mean of healthy subjects). About half (43) had impairment in one test, and 14 had impairments in 3 or more tests. The average FBI was higher among ALS patients with impairments in 3 or more tests (20.8 vs. 9.0, $p < 0.016$), but high FBI scores were only found in 5/14 of these ALS patients. Among patients who underwent detailed neuropsychological testing (6 with ALS-FTD, 8 with ALS-cognitive impairment but not dementia, and 7 with ALS and normal cognition), impairments in 2 or more tests is associated with 81.0% accuracy in differentiating between ALS-FTD and other ALS patients (compared to 61.9% using the mini-mental status examination). Impairments in confrontation naming were also common among ALS-FTD patients, and were associated with 90.5% diagnostic accuracy in differentiating between ALS-FTD and other ALS patients.

Discussion and conclusion: Executive dysfunction is common in ALS patients, and a brief cognitive assessment can be performed during a busy multi-disciplinary ALS clinic to detect subtle cognitive impairments. Impairments in confrontation naming could occur without fluency impairments, and may be more accurate in predicting extra-motor cortical involvement of TDP-43 pathology in ALS.

C34 THE EYES MAY HAVE IT. EYE-TRACKING AS A BIOMARKER FOR EXTRAMOTOR PATHOLOGY IN ALS

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Keywords: eye-tracking, extramotor involvement, biomarker

Background: ALS is a multiple system cerebral neurodegeneration for which there is no fully validated diagnostic or monitoring biomarker. The most consistent cognitive deficits relate to executive dysfunction, but traditional neuropsychological assessment is frequently lengthy and may not be a practical or available option for patients, especially those in the later stages of the disease. The oculomotor system involves a network of brain regions that includes areas of the frontal lobes consistently implicated in ALS extramotor pathology. The relative sparing of the brainstem nuclear oculomotor pathways throughout the disease course may be exploited to probe supranuclear pathology by studying oculomotor tasks that depend on intact higher pathways.

Objectives: To explore the potential of eye-tracking as a source of biomarkers for extramotor pathology in ALS.

Methods: Heterogeneous ALS patients ($n = 31$), PLS patients ($n = 5$) and age-similar healthy controls ($n = 26$) were tested using the EyeLink® eye-tracker. The assessment battery included a prosaccade ("look towards"), antisaccade ("look away"), word and picture-cued visual search tasks, and a novel oculomotor version of the Trail-making test (and where possible, the traditional written version for comparison). Participants were also assessed using the revised Addenbrooke's Cognitive Examination (ACE-R).

Results: Both antisaccade latency and error rates were markedly increased in both ALS and PLS patients ($p < 0.001$) in the absence of a significant difference in either the prosaccade velocity ($p = 0.5$) or latency ($p = 0.7$), suggesting supranuclear dysfunction. Antisaccade error rates in PLS patients were significantly higher than in ALS patients ($p < 0.05$). Both word and picture-cued visual searches were significantly slower in ALS and PLS patients ($p < 0.05$), supporting frontostriatal involvement. The number of fixations taken before arriving at the target was higher in the word- cued search ($p < 0.05$). A strong correlation was seen between the written and oculomotor Trail- making test performance in both patients and controls ($r^2 = 0.7$, $p = 0.01$). Total time to completion of the oculomotor Trail making test, and the number of fixations involved, was significantly higher in both patient groups, with a marked difference between the Trail B versus Trail A performance ($p < 0.001$), in keeping with executive dysfunction.

Discussion: The antisaccade task and oculomotor Trail-making test were significantly impaired in a heterogeneous group of ALS and PLS patients despite normal prosaccade velocity and latency. This is likely to reflect extramotor pathology. The demonstration of greater impairment in those with PLS raises new questions about extramotor involvement in this subgroup. Potentially, an antisaccade task derived from a portable saccadometer could be easily introduced within neurological centres as part of the diagnostic work-up as well as subsequent monitoring of ALS and PLS patients.

Conclusions: Eye-tracking has significant potential as an objective, quantifiable biomarker of extramotor pathology. Longitudinal validation with structural and functional MRI measures of cerebral involvement, as well as histopathology, is now required.

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SESSION 6A BEYOND GUAM: NEW ASPECTS OF THE BMAA HYPOTHESIS

C35 GUAM ALS/PDC: A DOORWAY TO UNDERSTANDING SPORADIC ALS?

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Keywords: Guam, cyanobacteria, BMAA

BMAA in Guam: Studies of ALS/PDC foci in Guam, Japan's Kii Peninsula, and West Papua, have identified a variety of potential genetic and environmental risk factors. There is renewed interest in β-N-methylamino-L-alanine (BMAA), a neurotoxic amino acid originally isolated from Guam cycad seeds, as a possible trigger for sporadic ALS in gene/environment interactions (1).

BMAA occurs both as a free amino acid and as a protein-bound amino acid in multiple components of the traditional Chamorro diet, including tortillas, dumplings, and stews made from cycad seeds and the flesh of wild or feral animals that feed on cycad seeds (2,3). Protein-bound BMAA occurs in higher concentrations than the free amino acid in cycad gametophytes and other tissues. Washing cycad chips with water fails to remove bound BMAA, causing previous investigators to underestimate BMAA concentrations in cycad flour (4).

BMAA in other ecosystems: BMAA is produced by endosymbiotic cyanobacteria of the genus *Nostoc* in specialized, positively geotropic roots of cycads (5). BMAA is also produced by other cyanobacterial taxa, suggesting that exposure to BMAA may occur far from Guam (6). Double-blinded analyses of post mortem brain tissues detected BMAA in North American Alzheimer's patients but not healthy controls (2). This result was replicated by researchers at the Miami Brain Bank who also found BMAA in ALS patients but generally not in Huntington's patients or in healthy controls (7). Investigators in Sweden and Florida found BMAA accumulating in ascending trophic levels in marine ecosystems (8,9). Investigators in New Hampshire and France suggest that cyanobacterially-contaminated water and shellfish may be linked to clusters of sporadic ALS (10, W. Camu in press).

Neurotoxicity to motor neurons: BMAA has effects on all of the main types of glutamate receptors: NMDA, AMPA/kainate, and metabotropic receptors, particularly in the presence of bicarbonate (11,12). Selective toxicity of BMAA at concentrations of 30 μM to sub-populations of motor neurons distinguished by the presence of NADPH-diaphorase is mediated by AMPA/kainate receptors. BMAA also acts on the cystine/glutamate antiporter (system x_c^-), where it induces oxidative stress and glutamate release. At concentrations of 10 μM, BMAA potentiates neuronal injury induced by exposure to other neurotoxins, including amyloid-β or 1-methyl-4-phenylpyridinium ion (MPP+), which are used in models of Alzheimer's and Parkinson's disease respectively (11).

Protein misincorporation: New findings to be reported at this meeting by Australian researchers show that BMAA is misincorporated into human proteins through mischarged tRNA, causing changes in protein confirmation and collapse. Swedish and French investigators report that BMAA binds to melanin in animal models (13,14).

New approaches: Studies of BMAA suggest new approaches to ALS prevention and therapy, including a drug currently in Phase II human clinical trials (15).

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C36 AN ENVIRONMENTAL NEUROTOXIN, BMAA, IN GUAM

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Keywords: BMAA, neurotoxin, environment

Background: ALS/PDC in Guam has been examined from many different research perspectives and the idea of an environmental link to the disease is long-standing. The data demonstrating that the Chamorro diet is rich in the neurotoxin β-methylamino-L-alanine (BMAA) suggest a mechanism for chronic dietary exposure in traditional villages. Cyanobacterial symbionts in specialized cycad roots produce BMAA. Free-

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living cyanobacteria from habitats worldwide also produce BMAA. The hypothesis that cyanobacterially produced BMAA can trigger sporadic ALS in vulnerable individuals is testable and has the potential to lead to target-based therapy.

Objectives: We established analytical techniques to separate BMAA from related compounds and to quantify BMAA concentrations in a variety of complex physiological matrices including cyanobacteria, plant, animal, and human tissues.

Methods: Using AQC precolumn derivatization with HPLC-FD, UPLC-UV, LC/MS, and LC/MS/MS detection, we analytically separated BMAA from other diamino acids and amides particularly those that might occur in complex physiological matrices, such as 2,6-diaminopimelic acid. An amino acid analyzer which separates underivatized BMAA using an ion-exchange method with post-column colorimetric detection employing ninhydrin was also used. Free amino acids are analyzed using a TCA extraction method and compared with water, methanol, and acetone extractions. Protein-bound amino acids were analyzed using standard hydrolysis techniques and with enzymatic degradation (glucosidases and Pronase). Verification across multiple instruments, by multiple investigators in different laboratories, using different techniques provides increased confidence in the determination and quantification of BMAA, particularly within complex physiological matrices.

Results: We found that standard methods of amino acid analysis clearly distinguish BMAA from other diamino acids and amides. Free and protein-bound BMAA was detected throughout the tissues of 9 Guamanian flying fox specimens, *Pteropus mariannus mariannus*, including internal organs, muscles, skin, hair and in the skin, hair, and in the liver of 3 *Pteropus mariannus yapensis* but not in the kidney or muscle. In 2 dried specimens of *Pteropus tonganus*, BMAA was not detected. Hydrolyzed protein samples released higher concentrations of BMAA than free-BMAA extractions in washed cycad flour samples. Enzyme degradation experiments demonstrated that BMAA is released with Pronase but not with glucosidases in cycad gametophyte tissues.

Conclusions: Current analytical methods to quantify BMAA using AQC derivatization reliably separate BMAA from other diamino acids and amides. BMAA is associated with both the free and protein fraction of animal and plant tissues. Enzyme degradations suggest that BMAA is covalently bound to the insoluble seed material from *Cycas micronesica*: that it is not bound to the carbohydrate component, but is bound to the protein component of the seed. The current data support the hypothesis that cyanobacterially produced BMAA may be a trigger for sporadic ALS in vulnerable individuals.

C37 DETECTION OF BMAA IN THE MARINE ENVIRONMENT OF A SPORADIC ALS CLUSTER IN SOUTHERN FRANCE

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Keywords: cluster, BMAA, standardized incidence rate

Background: The causes of sporadic ALS (SALS) remain unknown in the vast majority of the cases but several environmental studies have pointed to the role of exposure to BMAA as a potential risk factor for developing ALS. Recent research determined that BMAA, produced by cyanobacteria, is present in marine areas with cyanobacterial blooms and can bioaccumulate from invertebrates to animals of higher trophic levels, ending in human consumption.

Objectives: To identify potential SALS clusters from Languedoc-Roussillon and investigate the existence of BMAA in the surrounding area.

Methods: Potential SALS clusters were identified from our database from 1994 and 2010. Our cases were then studied with the standardized incidence ratio (SIR) method. Environmental investigations were done in the most important cluster with local collection of environmental samples. These samples were analysed for BMAA and its neurotoxic isomer 2,4-DAB.

Results: From the several potential clusters identified in our data base, 9 had a significant SIR. Two of them, Mèze and Balaruc, corresponded to cities surrounding the Thau Lagoon with an SIR of 3.31 and 3.47, respectively. In these areas, food intake as well as the economic structure were focused on the culture and collection of molluscs. Local investigations found that oysters from the lagoon were macroscopically positive for phycocyanin, a marker for the presence of cyanobacteria. LC/MS/MS analysis of mussels and oysters, collected at different periods, showed BMAA concentrations ranging from 1.83 to 6.04 µg/g in mussels and from 0.52 to 2.11 µg/g in oysters, while no difference in DAB concentrations could be noted between oysters and mussels. The highest concentrations of BMAA were noted during summertime when the highest picocyanobacterial abundances have been recorded. Otherwise, seasons did not apparently influence DAB levels.

Discussion: BMAA is found in invertebrates of the Thau Lagoon, an area in which their consumption by indigenous populations is particularly important all-year-long. If the relationship with the occurrence of SALS cannot be formally ascertained, it is nevertheless tempting to speculate that populations from the cluster are at high risk of BMAA intake as they still eat shellfish during summer, unlike other people. The highest cyanobacteria densities are regularly observed during summer in Thau lagoon. Thus, this period could potentially present risks because of the transfer of BMAA in the trophic chain via the microbial loop. We plan to analyze brain tissues from deceased patients to determine whether their content in BMAA is high as it has been demonstrated in patients from other areas such as Guam or Florida.

C38 THE CYANOBACTERIA-DERIVED NEUROTOXIN BMAA CAN BE INCORPORATED INTO CELL PROTEINS AND COULD THUS BE AN ENVIRONMENTAL TRIGGER FOR ALS AND OTHER NEUROLOGICAL DISEASES ASSOCIATED WITH PROTEIN MISFOLDING

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Keywords: neurotoxin, protein aggregation, BMAA

Background: Cyanobacteria (blue-green algae) are ubiquitously distributed in fresh water and marine environments. β-methylamino-L-alanine (BMAA), a non-protein amino acid produced by cyanobacteria has been linked to neurodegenerative disease in the South Pacific and to ALS and Alzheimer's disease (AD) in Canada and the USA (1,2). BMAA is bioconcentrated in aquatic species raising the possibility of 'silent' exposure to BMAA (3,4). BMAA is most commonly found in a protein-associated form [5]; the nature of this association is not known but there is evidence linking it to the chronic neurotoxicity of BMAA (6).

Hypothesis: BMAA is incorporated into cell proteins by protein synthesis in place of a 'protein' amino acid (7). The non-native proteins generated can misfold, inducing protein aggregation and a decline in cell function.

Objectives: To determine how BMAA is associated with proteins in mammalian cells *in vitro*.

Methods: Human fibroblasts (MRC-5) and neuroblastoma cells (SH-SY5Y) were exposed to culture medium containing ^{3}H-BMAA, and levels of intracellular free and protein-associated ^{3}H-BMAA quantified under a range of culture conditions. Release of ^{3}H-BMAA from the isolated cell proteins was then examined.

Results: Up to 10% of intracellular ^{3}H-BMAA was associated with cell proteins. The association between BMAA and cell proteins *in vitro* was both time and concentration dependent. Blocking protein synthesis using cycloheximide or increasing the concentration of protein amino acids in the culture medium, prevented any association between ^{3}H-BMAA and proteins. ^{3}H-BMAA could not be released from the isolated cell proteins by SDS, DTT or heat but could be released by proteolysis or acid hydrolysis.

Discussion: Release of ^{3}H-BMAA from cell proteins required cleavage of peptide bonds by proteolysis or hydrolysis, providing evidence that ^{3}H-BMAA was incorporated into proteins. The inclusion of a protein synthesis inhibitor in the culture medium prevented any association between ^{3}H-BMAA and proteins, confirming that incorporation was a protein synthesis dependent process. The extent of incorporation of ^{3}H-BMAA into protein was dependent on the level of amino acids present in the culture medium confirming direct competition between protein amino acids and BMAA for incorporation into protein.

Conclusions: BMAA, due to its ability to be incorporated into proteins by mammalian cells, could be an environmental trigger which promotes protein misfolding in genetically susceptible individuals.

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C39 TRACKING BRAIN UPTAKE AND PROTEIN INCORPORATION OF CYANOBACTERIAL TOXIN BMAA

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Keywords: beta-N-methylamino-L-alanine (BMAA), Parkinsonism-Dementia Complex (PDC), environmental toxins

Beta-*N*-methylamino-L-alanine (BMAA) is a neurotoxic non-protein amino acid. We and others have measured BMAA as a free and protein-bound amino acid in brains of Alzheimer's Disease (AD), Amyotrophic Lateral Sclerosis (ALS), and Guamanian ALS/Parkinsonism Dementia Complex (ALS-PDC) patients. A reservoir of BMAA misincorporated into proteins may function as a slow toxin that with continuous environmental exposure accumulates in brain. We report here on the biodistribution and time course of protein incorporation of custom synthesized [^{14}C]-L-BMAA in adult male C57 mice following single dose intravenous (i.v.) administration. The uptake of [^{14}C]-L-BMAA in brain was determined over time and quantified in soluble and protein fractions. The observed accumulation of radiolabeled BMAA following i.v. administration demonstrated that BMAA is taken up into the brain most likely through amino acid transport across the blood brain barrier. Accumulation of BMAA in brain was time dependent with increasing percentages of the total radiotracer occurring in the protein fraction at later time points. We observed over 70% of the radiotracer dose of BMAA incorporated into proteins at 3 days, suggesting an efficient transfer of BMAA from free to protein bound fractions. The uptake of BMAA and shift from the free to the protein bound fraction appeared to track the known rates of cerebral protein biosynthesis. *Ex vivo* autoradiography showed a relatively homogeneous distribution in brain at early time points after injection with increased BMAA labeling seen in the ventricles and choroid plexus. The regional pattern of BMAA uptake to the cerebral cortex and subcortical areas demonstrated that grey matter was elevated compared to white matter brain structures. These results demonstrate that the non-protein amino acid, BMAA, can be misincorporated into brain proteins and is in support of the toxic reservoir hypothesis. Environmental exposure to BMAA from the aquatic food web may lead to increased brain uptake and protein incorporation over time. Misincorporation of BMAA into cerebral proteins may alter normal function, folding and proteosomal degradation, increasing the risk for neurodegenerative diseases that affect aging populations.

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SESSION 6B EPIDEMIOLOGY

C40 EXOGENOUS RISK FACTORS IN ALS: A POPULATION-BASED CASE-CONTROL STUDY

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Keywords: risk factors, occupation, physical activity

Background: Sporadic amyotrophic lateral sclerosis (ALS) is probably caused by multiple genetic and environmental factors causing motor neuron degeneration. Although environmental risk factors have been extensively studied in ALS, most environmental risk factors are still unknown. Systematic reviews of the literature suggest this may be due to limitations in study design: most risk factor studies had a hospital-based study design, which introduces the risk of referral bias. This source of bias can be eliminated in a population-based case-control study, which enables the provision of class I evidence according to the Armon criteria (1) for exogenous risk factor studies in ALS.

Objectives: To determine the association between ALS and multiple exogenous factors: smoking; alcohol; education; medical history; medication use; nutrition; family history; hormonal factors; occupational history; occupational exposures (pesticides, metals, electrical accidents, etc.); physical activity.

Methods: A population based study has been performed in the Netherlands between January 2006 and June 2011 (mean population 16,426,273; area 41,528 km^2). Patients were ascertained from five sources. Diagnosis was made according to the El Escorial criteria.

700 incident sporadic ALS patients and 2100 controls filled in questionnaires to obtain data about exogenous factors.

Results: Multivariate analyses showed an increased risk of ALS in current smokers (OR 1.38; $p = 0.04$). Current smoking was also associated with shorter survival (hazard ratio of 1.51 ($p = 0.02$) adjusted for vital capacity, gender, age and site of onset). Current alcohol consumption was found to be an independent protective factor for ALS (OR = 0.52; $p = 6.6 \times 10^{-5}$), but did not have an effect on survival.

Relatives of sporadic ALS patients had a mildly elevated risk of dementia (recurrence risk λ 1.16; 95% CI: 1.01-1.33). The risk of Parkinson Disease (PD) was not elevated (λ 1.14; 95% CI: 0.83-1.55). A reduced risk of vascular diseases was found in relatives of sporadic ALS patients (stroke: λ 0.94; 95% CI: 0.82-1.07 and myocardial infarction: λ 0.87; 95% CI: 0.76-0.98).

Longest job held in the agricultural sector is associated with an increased risk of developing ALS (OR 1.7; 95% CI: 1.01-3.00, $p = 0.045$) (adjusted for smoking, use of alcohol, and age). Last job held in the agricultural sector is associated with ALS as well (OR 1.8; 95%CI: 1.1-3.1, $p = 0.03$). Subsequently a Job Exposure Matrix (JEM) was used, which enables the linking of occupations to profiles of environmental exposures by providing semi-quantitative assessments of exogenous exposures for each occupation. Mean lifetime occupational exposures to chromium, nickel, diesel motor exhaust, and mine dust were significantly higher in patients compared with controls. Exposure to pesticides was not significantly increased in patients.

Results on the other exogenous factors will be presented, as well as the result of a multivariate analysis including all exogenous risk factors.

Discussion and conclusions: Cigarette smoking, occupation in the agricultural sector, and a low level of education are risk factors for ALS. Current smoking is associated with a worse prognosis, alcohol consumption reduces the risk of ALS.

Familial aggregation of ALS, dementia and PD is substantially lower than previously thought. The lowered risk of vascular diseases in relatives of ALS patients supports the view that a beneficial vascular risk profile increases ALS susceptibility.

More risk factors will be analyzed and presented at the symposium. The multivariate analysis, including all exogenous risk factors, will determine which risk factors are independently associated with ALS.

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C41 PREMORBID CARDIOVASCULAR FITNESS IS A RISK FACTOR FOR ALS: EVIDENCE FROM RECORD-LINKAGE STUDIES

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Keywords: fitness, athleticism, cardiovascular risk

Background: Those at risk of developing apparently sporadic ALS have not yet been defined as a population. There is a persistent but anecdotal observation that ALS patients arise from a 'fitter' population, and some have proposed greater physical activity as a risk factor (1). Either way, intuitively such a physical profile might be reflected in a reduced incidence of coronary heart disease (CHD) prior to the development of ALS. The number of individuals and time required for a prospective study of co-morbidities or physical activity in ALS is untenable. Even the most rigorous case-control

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studies suffer from the major issue of recall bias, and have had conflicting results to date (2–4).

Objectives: To study the incidence of CHD in relation to the later development of ALS.

Methods: A record-linkage study of two large databases of hospital admissions, the Oxford Record Linkage Study (ORLS) and an English national record-linkage dataset of Hospital Episode Statistics was undertaken. The ratio of the rate of ALS in people without a record of CHD, to the rate in people with a record of CHD was calculated, factoring out premature death in the non-CHD and CHD cohorts. Similar analysis for Parkinson's disease (PD) and multiple sclerosis (MS) was undertaken.

Results: In the English population, the rate ratio (RR) for ALS in the non-CHD cohort was 1.14 (95% CI 1.05-1.22); for PD it was 0.95 (95% CI 0.93-0.98); and for MS 0.95 (95% CI 0.88-1.04). The ORLS data yielded similar findings.

Discussion: Those without a record of CHD were at higher risk of ALS than those with CHD. The higher risk was not found for PD or MS. This provides indirect support for the assertion that ALS arises in a cardiovascularly fitter population. Whilst this might be a result of an altered metabolic or physical activity profile, it could equally reflect distinct motor neuronal network properties associated with improved physical performance in youth, but inherently vulnerable to the stochastic events of ageing.

Conclusions: Studies of the genetic, molecular, and neuronal substrates of physical fitness are now a priority for the ultimate goal of the primary prevention of sporadic ALS.

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C42 MAY ALS PHENOTYPE BE CONSIDERED A PART OF THE SPECTRUM OF PARANEOPLASTIC DISEASES?

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Keywords: cancer, risk factor, survival

Background: It is still controversial whether typical ALS in cancer patients can be considered a paraneoplastic disorder.

Aims: To determine whether patients with ALS have a higher than expected incidence of cancer and how the co-occurrence of cancer modifies ALS prognosis.

Methods: The study population were the 1260 ALS cases incident in Piemonte and Valle d'Aosta in the period 1995-2004. Patients were affected by definite, probable or probable laboratory-supported ALS. Only patients with cancers occurring in the 6 months before and after the onset of ALS were included. The odds ratio of having a cancer in ALS patients was calculated using as reference the incidence rate of cancers in the same area for the period 2004-2006 (www.cpo.it). Odds ratio were calculated by gender and 5-year age-classes.

Results: 46 ALS patients had a cancer in the 6 months preceding or following the onset of ALS (11 lung, 10 breast, 7 gastrointestinal tract, and 18 other sites). No differences in the age and site of onset and gender distribution were found between cancer and non-cancer ALS patients. Cancer in ALS was significantly more frequent than expected in both genders (men, OR 2.01; 99% C.I. 1.15-3.28; women 3.43; 1.78-5.95). The higher frequency of cancers in ALS patients was mostly due to lung cancers for both genders (men 4.35, 1.51-9.68; women 4.83, 0.58-14.97) and to breast cancers for women (6.16, 2.29-13.18). Cancer patients had a significantly shorter survival than non-cancer patients (median survival time, 1.8 vs. 2.4 years; $p = 0.01$), but this difference was mostly due to the very short survival of ALS patients with lung cancer (median survival time, 1.0 year).

Conclusions: Cancer, predominantly lung and breast cancer, has an incidence significantly higher than expected in ALS population. Our finding suggests a possible correlation between some cases of ALS and cancer. Patients with lung cancer have a worse clinical progression of ALS than other patients.

C43 EPIDEMIOLOGY OF AMYOTROPHIC LATERAL SCLEROSIS IN THE ISLAND OF IRELAND FROM 1995-2010

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Keywords: registry

Background: Population based registries which use multiple methods of ascertainment are held in Ireland and Northern Ireland. The registry in Ireland was established in 1995 and the Northern Irish registry was established in 2005.

Objectives: 1) To determine whether the prevalence of ALS in Ireland has changed between 2000 and 2011; 2) To compare epidemiological findings between the two registries for incident cases of ALS diagnosed between January 1st 2005 and December 31st 2010.

Methods: Review of incidence, prevalence and survival in a population based cohort collected prospectively. To complete objective two, data mining from existing ALS Registers was carried out.

Results: 1) Between 1995 and 2010, 1306 cases of ALS were diagnosed in the Republic of Ireland providing a crude incidence rate of 2.6 per 100,000.

Prevalence data from the Irish Registry for years 2000 and 2010 were compared. Despite the introduction of treatments including non invasive ventilation, the prevalent rates have remained unchanged over a 10 year period.

2) Between 2005-2010, 477 cases of ALS were diagnosed in Ireland and 200 cases were diagnosed in Northern Ireland, giving a crude incidence rate of 2.6 per 100,000 in Ireland and 2.5 per 100,000 in Northern Ireland. Clinical and demographic data

was compared between the two regions. Mean age at onset was 65, and the male to female ratio was 1.15 :1 in both cohorts.35% of patients had bulbar onset disease in both cohorts. The mean duration from first symptom to disease onset was 14 months.

Conclusion: Prevalence of ALS remains unchanged, suggesting that the natural history of ALS has not changed over a 10 year period.

Despite differences in health care systems, the incidence, prevalence and clinical features of ALS, and duration from first symptom to diagnosis are identical in the Republic and Northern Ireland. This indicates that both Registers are close to full ascertainment and that delays in ALS diagnosis are unlikely to be influenced by the type of healthcare provided. The only statistically significant difference was the age at onset for females, which was higher in Northern Ireland.

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SESSION 8A CELL STRESS MECHANISMS

C44 THE ROLE OF THE ER IN MOTOR NEURON DEGENERATION

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Keywords: ER stress, unfolded protein response, protein disulphide isomerase

There is now substantial evidence for abnormalities to the endoplasmic reticulum (ER) and Golgi apparatus in diseases affecting motor neurons. In ALS, ER stress is emerging as important cellular pathway to cell death. The ER is primarily responsible for the folding, post-translational modification and trafficking of transmembrane and secretory proteins. ER stress is triggered when misfolded proteins accumulate within the ER. This induces the unfolded protein response (UPR): specific signalling pathways which aim to restore cellular homeostasis. However prolonged UPR induces apoptotic cell death. Many cellular insults lead to increased protein misfolding within the ER, including disturbances of the ER-Golgi vesicular transport pathway.

In this presentation I will review the increasing evidence implicating ER stress in the pathophysiology of ALS. Our laboratory previously demonstrated that the UPR is induced in lumbar spinal cords from transgenic $SOD1^{G93A}$ mice and sporadic ALS patients. Other studies have also shown that genetic manipulation of ER stress in SOD1 mice alters disease onset and progression. Furthermore, activation of the UPR is one of the earliest pathological events in affected motor neurons of $SOD1^{G93A}$ mice, and it is specific to those cells which degenerate first. Mutations to other proteins which modulate ER stress, such as VAPB and VCP, further implicate the UPR in ALS. More recently we demonstrated that ER stress is triggered by mutant TDP-43 and mutant FUS, thus implicating the UPR in both sporadic and other familial forms of disease.

The objective of our most recent work has been directed towards understanding the mechanisms by which the UPR is induced in ALS. Most of the proteins linked to ALS, including SOD1, are not usually associated with the ER, so it remains unclear how ER stress is triggered. However our most recent studies suggest that disruption to dynein-mediated protein trafficking between the ER and Golgi apparatus triggers ER stress in ALS. Disruption to ER-Golgi transport can also account for several other mechanisms implicated in neurodegeneration in ALS, including inhibition of axonal transport and fragmentation of the Golgi apparatus. Together these findings therefore implicate ER stress as an upstream mechanism in disease, and they provide a single mechanism to link the UPR to several other events previously described in ALS.

C45 DIFFERENT STRUCTURES OF SOD1 AGGREGATES CORRELATE WITH DIFFERENT DISEASE PHENOTYPES

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Keywords: aggregate, misfolded, prionoid

Background: Inclusions containing aggregated SOD1 are hallmarks of ALS in humans and transgenic models carrying mutant SOD1s (1), but are also seen in ALS patients lacking such mutations (2). This suggests that misfolded aggregation-prone monomeric, oligomeric and/or polymeric aggregated SOD1 species are critically involved in ALS pathogenesis.

Objectives: To map the structure of SOD1 aggregates in mice carrying human SOD1 mutants. From the results deduce nature of associations in oligomeric species and the processes involved in polymerization of SOD1 monomers, with the final goal of developing aggregation inhibitors.

Methods: Aggregates in serial dilutions of extracts of spinal cords and brains from terminal mice were captured on cellulose acetate filters in a 96-well dot-blot apparatus. Eight rabbit antibodies versus human SOD1 peptides covering $>90\%$ of the sequence were used for development of the filters similar to Western immunoblots. The antibodies react only with disordered SOD1 segments, and lack reactivity with ordered and native SOD1. In ordered and fibrous aggregates, the antibodies should lack reactivity with a β-strand backbone and react freely with the rest of the protein forming disordered fringes.

Results: The *in vivo* formed SOD1 aggregates are found to be highly ordered, with nearly equal antibody reactivity patterns (called type A) in the G93A, G85R, G127insTGGG models as well as in aged wild-type SOD1 transgenic mice. Many more aggregates were found in spinal cord than in brain, and none in liver and muscle. Remarkably, a distinct pattern (type B) was detected in D90A mice. SOD1 aggregates formed *in vitro* under a variety of conditions showed some resemblances with the *in vivo* aggregates, but were more heterogeneous. Aggregates from ventral horn from a patient carrying the G127insTGGG mutation were similar to those in corresponding mice.

Discussion: The *in vivo* crowding and proteostasis network (chaperones, transporters, redox regulators and degradation systems) shape highly ordered terminal aggregates, different from those formed *in vitro*. The antibody reactivity patterns

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suggest that some β-strands in native SOD1 are retained in the core fibrils, perhaps interacting via domain swaps. While most SOD1 variants seem to form 'type A' aggregates, D90A SOD1 formed different 'type B' aggregates. The disease phenotype provoked by this mutation is also deviant with very slow progression, sensory symptoms and bladder dysfunction, pointing at the possibility that different SOD1 'prionoid' species cause different diseases.

Conclusions: Highly ordered SOD1 aggregates are formed *in vivo*, differing between mutants. Correlations with disease phenotypes are found pointing at the possibility that ALS could be a SOD1 'prionoid' disease.

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C46 GLUTATHIONYLATION PROMOTES AGGREGATION OF SUPEROXIDE DISMUTASE IN ALS

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Keywords: glutathionylation, SOD1 aggregation, oxidative stress

Background: Mutation of the ubiquitous cytosolic enzyme Cu/Zn superoxide dismutase (SOD1) is hypothesized to cause familial amyotrophic lateral sclerosis (FALS) through structural destabilization leading to misfolding and aggregation. Considering the late onset of symptoms as well as the phenotypic variability among patients with identical SOD1 mutations, it is clear that non-genetic factor(s) impact ALS etiology and disease progression.

Objectives: Our objective was to examine the effect of Cys-111 glutathionylation, a physiologically prevalent post-translational oxidative modification, on the stabilities of wild type SOD1 and two phenotypically diverse FALS mutants, A4V and I112T.

Methods: We used size-exclusion chromatography for separation of modified SOD1 species from the unmodified ones, and to determine the dissociation constant K_d of wild type and the mutants. We use surface plasmon resonance to determine the kinetic rate constants for SOD1 dissociation. We use computational modeling to determine the structural implications of the SOD1 glutathionylation.

Results: Glutathionylation results in profound destabilization of $SOD1^{WT}$ dimers, increasing the equilibrium dissociation constant K_d to ~10-20 μM, comparable to that of the aggressive A4V mutant. $SOD1^{A4V}$ is further destabilized by glutathionylation, experiencing an approximately 30-fold increase in K_d. Dissociation kinetics of glutathionylated $SOD1^{WT}$ and $SOD1^{A4V}$ are unchanged, as measured by surface plasmon resonance, indicating that glutathionylation destabilizes these variants by decreasing association rate. In contrast, $SOD1^{I112T}$ has a modestly increased dissociation rate but no change in K_d when glutathionylated. Using computational structural modeling, we show that the distinct effects of glutathionylation on different SOD1 variants correspond to changes in composition of the dimer interface.

Discussion: The link between SOD1 mutations, protein aggregation, and FALS is not fully understood, but there are multiple reports showing that dimer dissociation is an early event during SOD1 aggregation. Our experimental and computational results show that Cys-111 glutathionylation induces structural rearrangements that modulate stability of both wild type and FALS mutant SOD1. Our finding that modifications can significantly facilitate SOD1 dimer dissociation (up to 1,000x fold) suggests a possible link between the normal characteristics of SOD1 and its role in FALS.

Conclusions: Protein glutathionylation is associated with redox regulation. The distinct sensitivities of SOD1 variants to glutathionylation, a modification that acts in part as a coping mechanism for oxidative stress, suggest a novel mode by which redox regulation and aggregation propensity interact in ALS.

C47 COMMON MOLECULAR PROFILE OF MISFOLDED SOD1 IN VARIOUS ALS MURINE MODELS

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Keywords: misfolded SOD1, oligomers, crosslink

Background: There should be one common mechanism by which the over 160 known mutations in SOD1 cause ALS. The only protein form common to all mutant SOD1s is a misfolded SOD1 species since variants exists (including truncated forms) that are incapable of folding to the native structure. Small amounts of misfolded SOD1 are a common denominator among murine ALS models expressing widely different forms of mutant SOD1s (1). Evidence for the involvement of misfolded SOD1 in sporadic ALS is emerging with SOD1 positive inclusions found in all types of ALS (2). We have developed a sensitive ELISA to measure low levels of misfolded SOD1 (misELISA) by the use of antibodies specific for misfolded SOD1 (3).

Objectives: To examine in detail the misfolded SOD1 protein in spinal cords from five strains of transgenic mice carrying different SOD1 variants. Mice expressing stable forms of human SOD1 (wt-hSOD1, G93A and D90A) are compared to mice expressing unstable variants (G85R and G127insTGGG)

Methods: Spinal cord extracts were made from fresh non-frozen tissues of pre-symptomatic mice (around 100 days old, n = 3 for each strain). The extracts were separated by size exclusion chromatography (SEC) and eluted fractions analyzed for total SOD1 by Western immunoblots and for misfolded SOD1 by misELISA. Parts of eluted fractions from some SECs were crosslinked to reveal any protein-protein interactions including oligomerizations.

Results: The same pattern of misfolded SOD1 was found in mice expressing widely different SOD1 variants. In all mice examined, the misfolded SOD1 was eluted from SEC as two

main peaks with higher apparent molecular weight than the normal SOD1 dimer. The molecular weights of these peaks were estimated to be 60 kDa and 200 kDa by comparison with calibrators of known molecular weight. By crosslinking, the misfolded SOD1 in the 60 kDa peak was shown to be monomeric while the SOD1 in the 200 kDa peak was composed of at least two oligomeric forms or bound to other cellular components.

Discussion: The main form of misfolded SOD1 in different transgenic mice is monomeric but with a significantly enlarged hydrodynamic radius which results in early SEC elution. The misfolded SOD1 with higher molecular weight are likely bound to chaperones but oligomeric forms may also exist. Misfolded soluble SOD1 is highly likely to be a precursor to large and smaller aggregates, the latter recently shown to facilitate spread of cytotoxicity in a prionoid manner.

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C48 A SEEDED AGGREGATION OF TDP-43 REPRODUCES THE INTRACELLULAR FORMATION OF SARKOSYL-INSOLUBLE INCLUSIONS

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Keywords: TDP-43, seeding, protein fibril

Background: A DNA/RNA-binding protein, TDP-43, was identified as a constituent of inclusions in most cases of sporadic ALS. Under pathological conditions, TDP-43 proteins form aggregates that are resistant to be solubilized by a detergent, sarkosyl. Simple overexpression of TDP-43 and its variants in cells have been reported to induce the formation of inclusions, but no cultured cell models are currently available in which sarkosyl-insoluble TDP-43 aggregates are reproduced.

Pathological TDP-43 aggregates are known to possess amyloid-like fibrillar morphologies. In general, formation of amyloid-like aggregates is accelerated by a seeding reaction, in which a pre-formed fibril functions as a structural template upon the recruitment of soluble protein into insoluble fibrils. While a seeded aggregation of pathogenic proteins has been proposed in several other neurodegenerative diseases, it remains unknown whether a seeding mechanism describes formation of sarkosyl-insoluble TDP-43 aggregates in cells.

Objectives: To understand a molecular mechanism of intracellular TDP-43 aggregation, we have first characterized TDP-43 fibrils *in vitro* and then examined the seeding ability of those *in vitro* fibrils to trigger the formation of sarkosyl-insoluble TDP-43 aggregates in cells.

Methods and results: Overexpression of TDP-43 in *E.coli* has led to the formation of inclusion bodies, which can be resolubilized with guanidine hydrochloride. Dilution of the guanidine hydrochloride concentration made it possible to refold our *in vitro* TDP-43; indeed, a filter-binding assay showed that refolded TDP-43 preferentially recognizes TG repeats of single-stranded DNA. These refolded and functional TDP-43 proteins were found to form sarkosyl-insoluble aggregates by constant agitation at 37 °C overnight. In addition, fibrillar morphologies of *in vitro* TDP-43 aggregates were confirmed by an electron microscopy.

TDP-43 fibrils *in vitro* were then transduced into HEK293T cells by using the BioPORTER protein delivery reagent. A TDP-43 variant expressed in a cell is C-terminally fused with an HA tag (TDP-43-HA), which discriminates intracellularly expressed TDP-43-HA from exogenously added TDP-43 fibrils. When *in vitro* TDP-43 fibrils were transduced into cells, we have found that intracellular TDP-43-HA forms sarkosyl-insoluble aggregates.

Discussion and conclusions: TDP-43 fibrils were found to exhibit a seeding activity *in vitro* and *in vivo*, which would reproduce the pathological formation of Sarkosyl-insoluble TDP-43 inclusions in the cell. It remains controversial whether the aggregation of TDP-43 is a cause or a result of the disease; however, as recently proposed in the other neurodegenerative diseases, a seeding activity of TDP-43 proteins may contribute to the propagation of pathological changes with the progression of diseases.

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SESSION 8B INTERNATIONAL PERSPECTIVES ON CARE PRACTICE

C49 PALLIATIVE CARE IN ALS: CURRENT INTERNATIONAL GUIDELINES AND INITIATIVES

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Keywords: palliative care, guidelines

The neurodegenerative diseases are relentlessly progressive and in the absence of effective disease modifying therapies, the emphasis remains on symptom management and maintenance of quality of life for the patient and carer. Optimal management requires a palliative approach from diagnosis. Palliative care enhances quality of life of patients and their carers by managing medical symptoms while also addressing individual psychological social and spiritual needs. Emphasis is placed on patient autonomy and dignity.

Despite an international consensus that management of neurodegenerative disease should adopt a multidisciplinary approach, integration of palliative care into disease management varies considerably across health care systems. However, common themes and principles of engagement can be identified across different jurisdictions, and measurement systems have been established that can assess the impact of palliative care intervention. International consensus guidelines would assist in the development of a framework for active palliative care engagement in ALS and other neurodegenerative diseases.

C50 THE EFFECTIVENESS OF PALLIATIVE CARE IN THE LATER STAGES OF MND/ALS

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Keywords: palliative care, effectivenss, quality of life

Background: Although palliative care has been involved widely in the care of people with ALS / MND there has been little research showing the effectiveness of the involvement. Previous studies on multiple sclerosis have shown limited effect.

Objectives: Using a mixed methods approach to both assess the perceived needs of people with progressive neurological disease and ascertain if specialist palliative care would reduce these needs and improve the quality of life of patients.

Methods: A qualitative needs assessment of 22 people with progressive neurological disease - ALS/MND, multiple sclerosis and Parkinson's disease - was undertaken with patients, their carers and professionals. A quantitative explorative randomised control trial of specialist palliative care was undertaken with 50 patients, comparing the immediate provision of the service with standard care.

Results: The qualitative study showed that there were many problems experienced by people with ALS/ MND, in all areas of care - physical, psychosocial, and spiritual. These needs had been noted by the professionals and they were positive about a new service to improve the care offered.

The randomised trial showed that there was statistically significant improvement in quality of life, pain, breathlessness, sleep disturbance and intestinal symptoms for the larger group of people with progressive neurological disease.

Conclusions: People with ALS/MND have a high burden of symptoms and other issues - psychological, social and spiritual. Specialist palliative care appears to be helpful in addressing these areas and in improving overall quality of life.

C51 OPINIONS AND BEHAVIORS OF JAPANESE AND AMERICAN NEUROLOGISTS REGARDING TRACHEOSTOMY WITH INVASIVE VENTILATION (TIV)

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Keywords: tracheostomy, invasive ventilation, questionnaire

Background: Studies in the United States, Europe and Japan have found widely disparate rates of Tracheostomy with Invasive Ventilation (TIV) use between countries, between areas within countries, and even in the same hospital. Many contributory factors have been suggested, including the treating neurologist's opinions.

Objectives: To obtain neurologists' approach to TIV in ALS patients.

Methods: We conducted national surveys of American and Japanese neurologists who specialize in ALS, selected because American patients have among the lowest, and Japanese the highest rates of TIV. We are asking patients and caregivers parallel questions. Here we present selected findings from the neurologist surveys.

E-mail address lists of American neurologists were obtained from the Muscular Dystrophy Association and the ALS Association, and cover letters were sent with surveys as attachments. In Japan, surveys were mailed to neurologists at major institutions caring for ALS patients.

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Results: 100 American neurologists, living in 44 states, and 80 Japanese neurologists have responded to date. Although over half of each sample has practiced neurology for more than 20 years, Americans were far more specialized: 77% currently treat > 20 patients/year, vs. 11% of Japanese neurologists.

While no Americans said their role was to make treatment decisions for their patients and inform them what will be done, 21% of Japanese neurologists do so. When asked what proportion of their own patients got TIV, 5% of American and 36% of Japanese said more than one-quarter of their patients did so. When we asked whether they generally suggest and encourage use of TIV, 79% of Americans and 38% of Japanese said "never" or "almost never."

Finally, of the 90 American neurologists who responded, 70% said they had been asked by patients or family members to discontinue TIV, compared to 59% of the 51 Japanese who responded. This difference is not statistically significant. Americans all complied, usually after several explanatory discussions, sometimes with an Ethics Committee consultation. In Japan, withdrawal of permanent ventilation is subject to prosecution.

Despite wide divergence in the likelihood of recommending TIV, and having patients get TIV, there is a striking similarity among American and Japanese neurologists when asked whether, if they themselves got ALS, would they accept TIV. Only 7% of Americans and 14% of Japanese said "yes" or "probably yes," compared to 76% of Americans and 71% of Japanese who said "no" or "probably no."

Discussion and conclusions: Compared to their Japanese colleagues, American neurologists do not encourage TIV, and have few patients who choose it. However, the large majority of both American and Japanese neurologists would decline TIV if they themselves were diagnosed with ALS. This surprising convergence of personal preferences suggests that other factors play a significant role in neurologists' behavior and recommendations regarding TIV.

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C52 COMPUTER ACCESSIBILITY: RECOMMENDING HEALTHCARE PROFESSIONALS AS A RESOURCE FOR INDIVIDUALS WITH ALS

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Keywords: computer access, assistive technology, healthcare professional

Background: New technologies are enabling individuals with motor disabilities to have alternative access to their computers. Though information regarding these technologies is available from a variety of sources, individuals with amyotrophic lateral sclerosis (ALS) present to the center with limited knowledge about these features.

Objectives: This study will collect information to evaluate the level of computer accessibility, familiarity and expectations of resources to determine if there is a need to provide education in this area.

Methods: A questionnaire was administered to individuals with ALS to establish their requirements and determine difficulties accessing their computers. They were asked to rate any difficulty they were having with their computer keyboard and/or mouse; their knowledge of the accessibility features currently available on the computer; and their knowledge of related software or hardware. Information was collected on how they have gained their existing knowledge and what they think the proper venue to obtain future education would be. The ALSFRS-R was recorded. In addition, the individuals rated their quality of life on a single item scale. The data was analyzed using the t-test ($p = 0.05$).

Results: A total of 40 subjects completed the questionnaire: 26 used the computer for pleasure only, none for work only, 13 for both, and one was unable to use the computer.

Only 7% of the subjects reported they never had difficulty with either the keyboard or the mouse. Over 60% of subjects indicated that their computer use was limited by accessibility. In reference to the accessibility features, 30% of subjects indicated 'Good' to 'Excellent' knowledge while 70% related that they had 'Fair' to 'No' knowledge.

Fifteen subjects reported no knowledge of accessibility features and 25 reported at least some knowledge. Of these 25 subjects, 60% indicated that they had gained their knowledge on their own. Only 15% of people reported a healthcare professional was involved as one of their resources, yet 72% of people indicated that they would expect a healthcare professional to be a resource. In addition, individuals with reduced hand function as measured by the ALSFRS-R score were more likely to indicate that a healthcare professional should be the source for information ($p = 0.02$).

Discussion and conclusions: Subjects reported a decrease in computer use as difficulty with accessibility increased. There was a high percentage of people with little or no knowledge of accessibility features (70%) and there was no consistent reliable resource. The majority of subjects indicated they would look to a healthcare professional as a resource. This is an unfulfilled need in this population and an assistive technology assessment would logically fit into the physical/occupational therapy evaluation in the clinical setting. It is recommended that healthcare professionals take the steps necessary to become a computer access resource for individuals with ALS.

C53 WWW.ALSHOME.DE – SELF-ASSESSED ONLINE SYMPTOM MONITORING IN ALS

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Keywords: electronic self-assessment, online management, ALS-FRSr

Background: Self-assessment of symptom progression and quality of life in chronic diseases is of increasing importance in clinical research and specialised outpatient healthcare. Against this background, we developed the Internet portal www.ALShome.de which provides online access to the ALS Functional Rating Scale, revised (ALSFRSr) and other established self-assessment questionnaires dealing with e.g. dyspnoea and appetite. In home care, an Internet-based self-assessment is a possible perspective for the monitoring of ALS-associated symptoms.

Objectives: To survey web-based self-assessment of ALS symptoms using the ALSFRSr and other established self-assessment questionnaires.

Methods: www.ALShome.de was created as a secure and closed Internet portal for patients. The application was developed in c-sharp (c#) with the persistent data storage being realised via an MS-SQL database throughout. Data are captured by generic questionnaires, are visualised on the website and administered via a content management system (CMS). Patients are assigned a discretionary number of online visits in defined intervals. In a prospective, controlled and stratified study, patients conduct a self-assessment of ALS-associated symptoms besides routine outpatient visits and home care.

Result: After one year of inclusion 162 patients (50 female, 112 male) gave informed consent to this study. Correlation between baseline and first online ALSFRSr (mean interval: 8.8 days) was excellent with a coefficient of 0.96 ($p < 0.001$) and the agreement of both capturing methods (online vs. onsite) was very good with more than 95% of all pairs of measurements within limits of agreement. 75% of the patients who attended a follow up onsite visit after 3.5 months in average performed online self-assessments all along. Patients were interviewed about the time burden and the emotional and physical strain of web visits. More than 95% felt that they are not at all or only slightly affected by these aspects.

Discussion and conclusion: The web-based self-assessment of ALS symptoms in a home care environment complements the well-established application of the ALSFRSr in outpatient departments. The results of our study indicate the good feasibility of the ALSFRSr as an Internet-based patient reported outcome measurement (PRO) and hence other PROs for the vertical and longitudinal measurement of outcomes. The study supports the hypothesis that innovative elements of self-management perspectively gain significant relevance in outpatient care.

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SESSION 8C SURROGATE MARKERS

C54 NEUROPHYSIOLOGICAL TESTING: STEPS TOWARDS EARLIER DIAGNOSIS

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Keywords: EMG, spinal reflex hyperexcitability, cortical involvement

A focus on diagnostic methods for MND has assumed greater importance as attention has shifted to the need for early diagnosis so that therapies have a chance to arrest disease progress before the degenerative process has become too advanced. Traditionally neurophysiological testing has been used to confirm clinically detectable abnormalities and to exclude alternative diagnoses, but in the current era they are of greatest value when they reveal abnormalities that are not apparent clinically. The "Awaji" criteria assign importance to EMG abnormalities, and represent a major advance because subsequent studies have shown that their use leads to earlier detection or identification of MND/ALS. A minor complication is that upper motor neurone (UMN) lesions such as stroke are associated with lower motor neuron (LMN) abnormalities, but this can be readily recognised and excluded. The Awaji criteria focus on the LMN. Degeneration of the upper motor neuron may be as or more important in many patients with MND syndromes. Three approaches can be used to identify UMN dysfunction: the first involves the demonstration that the spinal reflex arc is intact, perhaps even hyperactive, in a patient with LMN abnormalities involving the test muscle. Here the H reflex can be recorded in normal subjects from most limb muscles during a voluntary contraction of that muscle. Its presence indicates an intact reflex arc, and its presence in certain muscles at rest (for example, tibialis anterior and thenar muscles) indicates that there is hyperreflexia. The second approach involves demonstrating that reflex activation of a paretic muscle is greater than can be achieved in a maximal voluntary contraction of that muscle, again for a muscle that has EMG evidence of LMN abnormalities. For example, with a paretic tibialis anterior greater activation of tibialis anterior by noxious mechanical stimulation of the sole of the foot or in the H reflex than can be achieved voluntarily indicates a loss of the descending voluntary drive on the spinal motor neuron pool. The third approach involves demonstrating cortical hyperexcitability using transcranial magnetic stimulation (specifically, decreased "short-interval intracortical inhibition" in a paired-pulse paradigm). This testing can reveal abnormalities that precede the development of symptoms and signs in patients with familial ALS and, on the other hand, the absence of such abnormalities in Motor Neuron Diseases without corticospinal involvement (such as Kennedy's disease).

C55 CORTICAL HYPEREXCITABILITY APPEARS INTRINSIC TO ALS

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Keywords: cortical hyperexcitability, cortical plasticity, neurodegeneration

Background: The pathophysiological mechanisms underlying the development of amyotrophic lateral sclerosis (ALS) remain to be fully elucidated.Cortical hyperexcitability appears to be an important mechanism given that cortical hyperexcitability appears to be an early feature of sporadic ALS, linked to peripheral neurodegeneration, and that it precedes the development of familial ALS. It has been argued, however, that these changes in cortical excitability represent plasticity of the motor cortex in response to peripheral neurodegeneration.

Objective: Consequently, the aim of the present study was to determine whether cortical hyperexcitability is intrinsic process to ALS, or whether it simply represents cortical plastic changes.

Methods: Utilising the paired-pulse threshold tracking transcranial magnetic stimulation (TTTMS) technique, cortical excitability was prospectively assessed in a cohort of 156 consecutive patients with neuromuscular symptoms (104 ALS and 52 lower motor neuron syndrome, non-ALS syndrome, NALS). Results were compared to 62 healthy controls.The motor cortex was assessed using a 90 mm circular coil, with the motor evoked potential recorded over the abductor pollicis brevis (APB).

Results: The CMAP amplitude (ALS 5.6 ± 0.4 mV; NALS 7.8 ± 0.4 mV; controls 10.2 ± 0.4 mV, $P < 0.0001$) and neurophysiological index (ALS 0.6 ± 0.1; NALS 1.4 ± 0.1; controls 2.5 ± 0.1, $P < 0.0001$) were significantly reduced in ALS and NALS syndrome patients when compared to controls, indicating a comparable degree of peripheral disease burden in ALS and NALS syndrome patients. Cortical excitability studies disclosed a significant reduction in short interval intracortical inhibition (SICI) in ALS patients ($2.4 \pm 0.9\%$) when compared to NALS syndrome patients ($8.7 \pm 0.8\%$) and healthy controls ($10.6 \pm 0.8\%$, $F = 23.3$, $P < 0.0001$). Subgroup analysis revealed that although SICI reduction was a uniform finding in ALS, it was greater in ALS patients with less severe disease (SICI ALS CMAP > 4.5 mV $0.1 \pm 1.3\%$; ALS CMAP < 4.5 mV $3.7 \pm 1.5\%$, $P < 0.05$), thereby reaffirming that SICI reduction occurs as an early feature in ALS. In addition, intracortical facilitation (ALS $-2.2 \pm 0.7\%$; NALS $-0.6 \pm 0.6\%$; controls $-0.4 \pm 0.8\%$, $P < 0.05$) and MEP amplitude (ALS $38.5 \pm 2.7\%$; NALS $30.9 \pm 2.6\%$; controls $24.9 \pm 1.8\%$, $F = 9.6$, $P < 0.0001$) were significantly increased in ALS patients when compared to NALS syndrome patients and controls. Of further relevance, cortical silent period

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duration was reduced in ALS (180.9 ± 4.4 ms) when compared to NALS syndrome patients (210.0 ± 4.0 ms) and controls (210.3 ± 3.3 ms, $F = 12.7$, $P < 0.0001$).

Discussion and conclusions: The findings in the present study would seem to indicate that cortical hyperexcitability is a process intrinsic to ALS, clearly distinguishing ALS from the mimic disorders. In addition to suggesting that the threshold tracking TMS technique may prove useful as a diagnostic investigation for ALS, the present study would seem to argue against the notion that changes in cortical excitability simply represent cortical plasticity in ALS.

C56 MOTOR UNIT RECRUITMENT IN ALS AND OTHER UMN LESIONS

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Keywords: lower motor neuron, motor unit recruitment

Introduction: Motor unit potential (MUP) recruitment order and firing rate on volition depend on several different physiological factors, involving lower motor neuron (LMN) and upper motor neuron (UMN) interactions. The Henneman principle dictates early recruitment of type I motor units, but recruitment order is not studied during routine MUP analysis. Frequency variation among different MUPs is observed in routine EMG investigation.

Methods: We investigated potential MUP recruitment order and inter-MUP recruitment rate variation (coefficient of variation; CV) in controls and patients affected by different LMN and UMN disorders. Tibialis anterior muscle with preserved strength (normal walk on heels) was the only muscle investigated. We included 42 controls, 37 patients with ALS, 14 with progressive muscle atrophy (PMA), 37 with polyneuropathy, 23 with primary lateral sclerosis (PLS), and 14 with other thoracic/cervical spinal cord lesions (UMN lesion). MUPs were analysed as recorded from a minimum of 60 seconds steady, unquantified, mild muscular contraction, which usually activated 2 to 5 motor units. The same MUP was observed in at least 10 consecutive firings before analysis. Amplitude, area and duration of each MUP, order of recruitment and firing rate were assessed.

Results: In PMA, ALS and neuropathy larger MUP amplitudes, areas and durations were recorded, as expected, compared with the other groups. Correlation analysis did not reveal any specific abnormality of recruitment order in the different patient groups. The coefficient of variation for recruitment rate, applying the Bennett approach, as modified by Shafner and Sullivan, showed that no significant difference between subjects for PLS UMN lesion, but the other groups showed heterogeneity among individual subjects. However, the mean firing rate was similar in the different groups.

Discussion: Our data confirms reduced variation in MUP recruitment with UMN lesion. The limits of this abnormality need further evaluation, but we suggest that MUP recruitment could be useful as a tool to identify functional UMN impairment in the assessment of patients with ALS.

C57 MOTOR UNIT NUMBER INDEX (MUNIX) VERSUS MOTOR UNIT NUMBER ESTIMATION (MUNE): A DIRECT COMPARISON IN A LONGITUDINAL STUDY OF ALS PATIENTS

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Keywords: motor unit number index, motor unit number estimation

Background: Motor unit number index (MUNIX) is a surface EMG method that provides an index for the number of motor units and for motor unit size. MUNIX has a theoretical advantage over measuring the CMAP as MUNIX is not masked by reinnervation and it has the advantage over other motor unit number estimation (MUNE) methods as it is easier and quicker to perform. However, as MUNIX is an index and a gold standard is lacking, it is unknown how it is related to the actual number of motor units.

Objectives: To evaluate how MUNIX is related to the number of motor units as estimated by high-density surface EMG MUNE (HD-MUNE) and to determine the potential of MUNIX for monitoring disease progression in patients with ALS.

Methods: Both MUNIX and HD-MUNE of the thenar muscles were determined in 18 ALS patients and 24 healthy controls. Patients were measured at baseline, within two weeks (to assess reproducibility), and after 4 and 8 months. ALSFRS was scored and muscle strength tests were performed.

Results: HD-MUNE showed a slightly better reproducibility than MUNIX. There was a significant relation between MUNE and MUNIX in ALS patients but not in healthy controls. At baseline, MUNIX and MUNE were significantly lower in ALS patients than in healthy controls. Longitudinally, after 8 months, both MUNE and MUNIX of the ALS patients decreased significantly more as compared to MRC, ALSFRS and CMAP. There was no significant difference between the decline in MUNIX and HD-MUNE after 4 and 8 months.

Discussion and conclusion: In patients, MUNIX is related to the number of motor units as estimated by HD-MUNE and can be used to monitor disease progression in ALS. As MUNIX is much easier to perform as compared to HD-MUNE a multi-muscle approach seems feasible and can potentially further increase the sensitivity of the technique.

C58 RELATIONSHIP BETWEEN CLINICO-ELECTROPHYSIOLOGICAL DYSFUNCTION AND CERVICAL CORD ^{1}H-MAGNETIC RESONANCE SPECTROSCOPY IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: spinal cord magnetic resonance spectroscopy, clinico-electrophysiology, progression marker

Background: Amyotrophic lateral sclerosis (ALS) is a fatal disease characterized by upper and lower motor neuron degeneration. Previous studies of brain ^{1}H-magnetic resonance spectroscopy (MRS) revealed neuronal and axonal damage of the motor cortex, the corticospinal tract and the extra-motor cortex in ALS patients. Little is known about spinal cord ^{1}H-MRS in ALS patients.

Objectives: The present study aimed to examine whether neurological dysfunction and electrophysiological findings are related to metabolic changes on spinal cord ^{1}H-MRS in ALS patients.

Methods: Fifteen patients (8 men and 7 women) with definite or probable ALS fulfilled the El Escorial revised criteria, and 15 age-matched control subjects (8 men and 7 women) underwent cervical cord ^{1}H-MRS. A volume of interest with three dimensions of approximately $6.0 \times 8.0 \times 40.0$ mm (19.2 mL) was located along the main axis of the 1st to the 3rd segment of the cervical (C1-3) cord on T2-weighted images. Four signal amplitudes of N-acetyl-aspartate (NAA), choline-containing compounds (Cho), creatine plus phosphocreatine (Cr) and myo-Inositol (m-Ins) were measured. ALS functional rating scale (FRS) and forced vital capacity (FVC) were assessed every month. Electromyography was performed at the same period of C1-3 cord ^{1}H-MRS.

Results: NAA/Cr was decreased significantly ($p < 0.05$) and m-Ins/Cr was increased significantly ($p < 0.05$) in ALS patients compared to control subjects. NAA/m-Ins was correlated inversely with decline rates of monthly FRS and FVC. NAA/m-Ins was also linked inversely to electromyographic profiles of the motor nerve degeneration, including ongoing denervation and reduced amplitudes of compound muscle action potential in the upper limb muscles. NAA/Cr and m-Ins/Cr were preserved in two patients who had severe degree of bulbar palsy and mild degree of weakness and atrophy in the four limb muscles.

Discussion and conclusions: Neuronal degeneration, axonal loss and demyelination occur in ALS patients, leading to changes of several metabolites. Only one recent study of cervical cord ^{1}H-MRS has suggested reduction of NAA/Cr and NAA/m-Ins in ALS patients at 40% and 38%, respectively. NAA/m-Ins and NAA/Cho are linked to FVC. No significant relationship existed between those ratios and FRS (1). The present study indicated a significant decrease of NAA/Cr and a significant increase of m-Ins/Cr in ALS patients compared to controls. The inverse relationship was found between the NAA/m-Ins, FRS, FVC, and axonal damage in the upper limb muscles on electromyography. Otherwise, cervical cord ^{1}H-MRS did not reflect damage of the bulbar neurons. Thus, cervical cord ^{1}H-MRS could have benefits as a predictive marker for progression of limb and respiratory motor dysfunction in ALS patients. Further serial studies of cervical cord ^{1}H-MRS are needed to elucidate how the metabolic changes are associated with clinico-electrophysiological deterioration in ALS patients.

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SESSION 9A GENETICS

C59 DIVERSE ETIOLOGIES AND CONVERGENCE OF PATHOLOGY IN ALS

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Abstract not available.

C60 SIGMA RECEPTOR 1 MUTATIONS CAUSE FRONTOTEMPORAL LOBAR DEGENERATION–MOTOR NEURON DISEASE

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Keywords: genetics, FTLD, neuropathology

Pathological ubiquitinated inclusion bodies observed in frontotemporal lobar degeneration (FTLD), the most common cause of early-onset dementia, and motor neuron disease (MND) contain TDP-43 and/or FUS proteins, suggesting that there may be a shared etiology between these disorders. Using a large FTLD-MND pedigree that showed significant linkage to chromosome 9p, our objective was to identify the causative gene using positional cloning. We identified a mutation (c.672*51G>T) in the 3'-untranslated region (3'-UTR) of the sigma nonopioid intracellular receptor 1 (SIGMAR1) gene in affected individuals from the FTLD-MND pedigree. This mutation was not found in over 2,500 neurologically normal chromosomes (1). Mutation screening of 26 FTLD probands, negative for MAPT and GRN mutations, and 158 unrelated cases of familial presenile dementia, negative for known dementia mutations, identified two additional 3'-UTR mutations, c.672*26C>T and c.672*47G>A. Screening a further 119 neuropathologically diagnosed neurodegeneration cases for mutations in SIGMAR1 identified one patient with the corticobasal degeneration form of FTLD-tau, with both tau and TDP-43 immunoreactive pathology, who had a 21 bp deletion in the 3'UTR (c.672*800_*820del). The c.672*51G>T mutation significantly increased gene expression in a luciferase reporter assay by 1.4-fold, corresponding with a significant 1.5- to 2-fold change in the SIGMAR1 transcript or Sigma-1 protein in lymphocyte or brain tissue. The c.672*800_*820del deletion mutation also resulted in a 1.5-fold increase in gene expression. However, the c.672*26C>T and c.672*47G>A mutations decreased luciferase activity by 0.8-fold. Neuropathological characterization of the brains of affected individuals showed that SIGMAR1 mutation carriers displayed a unique pathology with cytoplasmic inclusions, immunopositive for either TDP-43 or FUS, but not Sigma-1. Overexpression of SIGMAR1 in cell lines and Western blot studies were performed to identify the pathological mechanism of the mutation. Overexpression of SIGMAR1 shunted TDP-43 and FUS from the nucleus to the cytoplasm by 2.3-fold and 5.2-fold, respectively. Treatment of cells with Sigma-1 ligands significantly altered translocation of TDP-43 with the agonist opipramol causing 1.5-fold increased levels, and the antagonists AC915 and haloperidol causing up to 2-fold decreased levels of cytoplasmic TDP-43. Mutations in the SIGMAR1 gene have been shown to be a cause of a neuropathologically unique form of FTLD-MND, as well as in other degenerative conditions such as the corticobasal degeneration form of FTLD-tau. However, mutations in the SIGMAR1 gene do not appear to explain all cases of chromosome 9p-linked FTLD or MND, suggesting that there are additional causal genes to be discovered. Our findings also suggest that Sigma-1 drugs have potential for the treatment of the TDP-43/FUS proteinopathies.

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C61 COMBINED GENOME-WIDE ANALYSIS IDENTIFIES UNC13A AND CHROMOSOME 9P21.1 AS SHARED LOCI FOR SUSCEPTIBILITY TO AMYOTROPHIC LATERAL SCLEROSIS AND FRONTOTEMPORAL LOBAR DEGENERATION

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Keywords: frontotemporal lobar degeneration, meta-analysis, UNC13A

Background: Overlap exists for patients with Amyotrophic Lateral Sclerosis (ALS) and patients with frontotemporal lobar degeneration (FTLD), both clinically and pathologically as well as genetically. About 5-10% of ALS patients are diagnosed with FTLD, while FTLD patients can develop motor neuron symptoms. Furthermore, TDP-43 inclusions have been found in the majority of ALS patients and in cases of FTLD (FTLD-TDP). Previously, linkage studies in families with both ALS and FTLD have identified a locus on chromosome 9p13-21.

Objectives: We hypothesized that ALS and FTLD may be part of a spectrum of neurodegenerative disease and we sought to identify a common genetic basis for this neurodegenerative disease.

Methods: We obtained genome-wide single nucleotide polymorphism (SNP) genotype data from previously published studies on ALS and FTLD-TDP and performed genome-wide imputation based on the HapMap3r2 reference. After quality control, genome-wide data for ~1.2M SNPs were available for nearly 4,400 ALS patients and over 13,000 controls and for 435 (pathology-proven) FTLD-TDP cases and 1,400 independent unaffected controls. Data were analyzed in a joint meta-analysis of both diseases and by using a conservative rank-products analysis, weighing ALS and FTLD sample sizes equally. Additionally, a subset of FTLD patients without motor neuron disease symptoms was analyzed.

Results: Joint analysis of ALS and FTLD identified SNPs in locus chromosome 9p21.1 (lowest $p = 2.6 \times 10^{-12}$) and SNP rs12608932 in gene *UNC13A* ($p = 1.0 \times 10^{-11}$) as shared genetic variants contributing to susceptibility to both neurodegenerative disorders. From the rank-products analysis we found the associations to be consistent. By analyzing a subset of FTLD patients without motor neuron disease signs, we found that individuals with ALS symptoms did not solely drive the signal for both loci.

Discussion and conclusions: Our study narrows the previously identified shared genetic locus on chromosome 9 and, additionally, implicates *UNC13A* as a shared risk locus further corroborating the role of *UNC13A* in neurodegeneration.

C62 POLYALANINE REPEAT EXPANSIONS IN NIPA1 ARE ASSOCIATED WITH ALS

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Keywords: NIPA1, polyalanine, repeat expansion

Introduction: Genetic risk factors are thought to play a major role in the pathogenesis of ALS.Recent research has described deletions in NIPA1 increasing susceptibility for this disease. Mutations in this gene are known to cause hereditary spastic paraparesis type 6. In this study we investigate whether missense mutations in NIPA1 and expansions in its polyalanine repeat might play a role in ALS as well.

Methods: DNA samples were collected from 2293 ALS patients and 2777 healthy controls, of three different populations (Dutch, Belgian, German). All exons of NIPA1 were sequenced and fragment analysis was performed to determine the polyalanine repeat length. Alleles were grouped according to their length, with short alleles consisting of < 12, intermediate and long alleles of 12-13 and > 13 alanines respectively.

Results: Sequencing revealed 7 missense mutations in patients and 6 in controls ($p = 0.59$). In all populations the long repeat occurred more frequently in patients, 2.82% compared to 1.80% in controls (OR = 1.63, $p = 0.0007$). Patients with these long alleles showed worse median survival (HR 1.58, $p = 0.0001$) and had an earlier disease onset (HR 1.39, $p = 0.005$).

Conclusion: We found that missense mutations in NIPA1 do not play a major role in ALS. Instead, we found that short polyalanine expansions in NIPA1 are associated with ALS. In addition, these expansions are associated with an earlier disease onset and worse prognosis. These results further underscore the role of NIPA1 in ALS pathogenesis.

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SESSION 9B INTERNATIONAL PERSPECTIVES ON CARE PRACTICE

C63 NOT ONLY MND: SELF-REPORTED CO-MORBIDITIES AND OTHER DIFFICULTIES THAT IMPACT ON THE NEED FOR HOME CARE SUPPORT AND ALLIED HEALTH SERVICES

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Keywords: co-morbidities, home care support, allied health services

Background: In 2006, a survey of the characteristics and support needs of adults living with Motor Neurone Disease (MND) and other neurodegenerative disorders was conducted in Western Australia (Giles and Lewin, 2008). The survey data provided self-reported information on co-morbidities and physical and mental health symptoms as well as respondents' functioning defined in terms of Lawton and Brody's (1969) Incidental Activities of Daily Living (IADL) and Barthel's Activities of Daily Living (ADL) (Collin, Wade, Davies, & Horne, 1988). Giles and Lewin used the IADL and ADL results to assign a level of dependency to each of the respondents. Home support needs for people with MND, disaggregated by level of dependency and type of support, were reported to the 2008 APF in Birmingham, UK.

Objectives: This paper will report on the development of a composite index to predict the need for home support and allied health services, for people with MND. A comparison will be made of the usefulness of this composite index compared with using broad levels of dependency.

Outcomes: The survey sample of people with MND (n = 56) had up to four co-morbidities. The most common of these were high blood pressure (15%), diabetes (12%), musculoskeletal problems (10%), depression/anxiety (8%) and cancer (7%). Difficulties related to physical and psychosocial symptoms were experienced to some degree by all people with MND in the sample.

The composite index includes number of co-morbidities as well as number of difficulties weighted according to how they are reported in the survey - mild, medium or extreme. These difficulties may be related to co-morbidities or to the underlying MND disorder. Alternatively, the number and severity of the difficulties may also be related to age or length of time since appearance of first symptoms. Hence these two additional variables are also incorporated into the composite index.

The index and suggestions for how it may be used, together with the results of the comparison analysis, will be described in the paper.

Recommendations to the field: Care pathways for people with MND should also consider co-morbidities and physical and psychosocial symptoms that could compound their needs for services, including allied health and home care support services. The composite index described in this paper can provide useful input to the development of these care pathways.

C64 INVESTIGATING ASPECTS OF SOCIAL COMMUNICATION IN AUSTRALIANS WITH MND

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Keywords: cognition, emotion, communication

Background: Cognitive and behavioural changes in people with Motor Neurone Disease (PwMND) without FTD are well established. While there has been extensive research into these areas, less research exists examining the impact of social communication changes, particularly the impact on caregivers. High level social communication is dependent upon a combination of factors, including emotion recognition, pragmatic language skills and social cognition. Impaired social communication may be seen as 'social inappropriateness' and has been found to be a significant factor in interpersonal relationships and quality of life.

Objectives: The current research aimed to prospectively investigate the nature of social communication deficits in Motor Neurone Disease (MND), by examining affect recognition, and also the recognition of sarcasm and sincerity. It was hypothesised that PwMND without dementia would perform worse than healthy controls, and that these changes would be associated with reduced empathy. The current research also aimed to examine the relationship between changes in social communication, and caregiver burden and psychological ill health.

Methods: 38 PwMND without dementia and their nominated caregiver, and 27 age- and sex-matched healthy controls and informants completed selected TASIT and CATS subtests, the LaTrobe Communication Questionnaire and an empathy questionnaire. Caregivers also completed caregiver burden and mood questionnaires. Data were analysed using non-parametric methods.

Results: In comparison to healthy controls, PwMND showed significant difficulty on a task requiring participants to interpret sincere and complex sarcastic interpersonal exchanges, particularly in the absence of contextual cues. When contextual cues were present, PwMND were able to accurately

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identify the basic affective states of others as effectively as healthy controls.

For PwMND, difficulties in both discriminating facial affect and recognizing contextual positive emotions were associated with increased anxiety and depression in carers. Behavioural changes such as increased apathy, disinhibition and executive dysfunction were also associated with increased carer burden and depression.

Overall caregivers of PwMND did not identify significant changes in everyday social communication and empathic concern, relative to controls.

Discussion and conclusions: Results demonstrated that PwMND without dementia do have difficulties with affect recognition when contextual cues are absent. While affect recognition was improved when PwMND were provided with context, higher level deficits in the recognition of sarcasm and sincerity in social situations remained. These impairments were present in the absence of noticeable changes in carer-rated everyday social communication and empathy. The current study has captured a cohort of PwMND who may be in the early stages of decline in their overall social communication. Given impaired social communication can negatively impact social relationships and may contribute to relationship stress and social isolation, early screening has the potential to provide more timely support to carers who are dealing with these changes.

C65 JUDGING A BOOK BY ITS COVER? WELL-BEING AND DECISIONS IN ALS

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Keywords: well-being, peer assessment, advanced directives

Background: Decisions to prolong or shorten life in fatal diseases like ALS are strongly influenced by healthy individuals, like caregivers and physicians. Furthermore, some researchers and clinicians suggest that ALS patients should decide ahead of time on advanced directives to circumvent any confounding effects of cognitive impairments on decision making in the course of ALS. It is not yet clear whether healthy individuals can correctly anticipate a patient's well-being and decisions.

Objectives: It was the aim of the study to determine the ability of healthy individuals (caregivers and aged matched healthy individuals) to anticipate a patient's well-being and his decisions.

Material and methods: Forty ALS patients and their caregivers and 110 age-matched healthy individuals were asked to judge factors of a patient with ALS: emotional well-being (depression and quality of life) and decisions to hasten death. Depression was determined with Allgemeiner Depressions Fragebogen, a German version of the Center for Epidemiological Studies Depression Scale (CES-D-Scale), and the ALS Depression Inventory 12 Items (ADI-12). Quality of life was determined with the Anamnestic comparative self assessment (ACSA). Attitudes toward hastened death were evaluated with the Schedule of Attitudes Toward Hastened Death (SAHD). All scales have been validated in ALS studies.

Patients judged their own well-being and decision status, the caregivers judged the well-being and decision status of the patient he/she is taking care of. Healthy individuals were asked to judge the well-being and decision status of a virtual patient. Additionally, caregivers and healthy individuals were asked to judge their own well-being. Cognition as a confounding factor for decision status was excluded in patient's group.

Results: Patients reported a good well-being and a low wish to hasten death. Caregivers and healthy individuals rated the patient's well-being significantly lower. The wish to hasten death was significantly lower in the patient group compared to what healthy individuals thought the/a patient would think. The rater's own well-being was a significantly better predictor of the peer assessement than the patient's well-being. Cognition was not associated with decision status in patients.

Discussion and conclusions: Patient's well-being was overall good. Healthy individuals were not correct in their assessment of a patient's well being. They rated the patient's well-being significantly lower. They draw their conclusions from their own well-being. Anticipating how a patient actually feels is more than challenging from the perspective of a healthy person. Advanced directives should be dynamically corrected to guarantee that the patient's will is as best met as possible when treatments are to be taken. Healthy individuals influencing decisions concerning the patient's life like caregivers, physicians and politicians should take these findings into consideration.

C66 THE IMPACT OF BEHAVIOURAL, BULBAR, RESPIRATORY AND MOTOR IMPAIRMENT ON ACTIVITIES OF DAILY LIVING IN MOTOR NEURON DISEASE

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Keywords: activities of daily living, behaviour, ALSFRS-R

Background: The nature of impairment in activities of daily living (ADLs) in motor neuron disease (MND) has been under investigated to date. Although ADL dependency is pervasive and present early in the disease, the main factors behind such disability have not being explored to date.

Objectives: The study aimed to investigate the contributions of behavioural (as measured by the Cambridge Behavioural Inventory Revised, CBI-R) and bulbar, respiratory and motor factors (as measured by the Amyotrophic Lateral Sclerosis Functional Rating Scale, ALSFRS-R) on ADL impairment.

Methods: A postal survey in New South Wales, Australia, included assessments of ADL, behavioural change (CBI-R, carer-based) and MND severity (ALSFRS-R).

Results: 82 patients were subdivided into bulbar (n = 23) and limb (n = 59) onset presentations. There were significant differences on ADL performance between limb and bulbar onset depending on ADL task. ADL disability correlated strongly with ALSFRS-R. More importantly, 57% of the variance on ADL scores was explained by a model combining behavioural and motor factors.

Discussion: Although primarily a condition affecting the motor system, MND also leads to changes in behaviour that, in turn, can also affect ADL performance.

Conclusions: This study confirms the progressive disabling nature of MND, which is strongly associated with disease severity and shows qualitative differences depending on onset presentation. Importantly, ADL disability is not only dependent on motor factors but also behavioural ones. These findings have clear implications for clinical intervention.

C67 PERSONA PROJECT - PERSONALITY CHANGES IN LATE STAGES OF ALS: AGGRESSIVENESS, SEXUALITY AND OBSESSIONS

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Keywords: personality assessment, aggressive behaviors, sexuality

Background: Scientific literature describes ALS patients as calm and pleasant, with a polite and accommodating attitude. However, studies about this topic are few and refer to early or middle stages of illness. Clinical evaluations suggest that, in late stages of illness, usually after tracheostomy, some patients may present a change of personality traits, in particular aggressive behaviors, obsessions and attitude toward sexuality. This clinical hypothesis has never been investigated.

Objective: The aim of this pilot study is to evaluate if personality traits of ALS patients during the late stages of illness, after tracheostomy, present significant changes related to aggressiveness, obsessions and attitude toward sexuality.

Method: We recruited 15 ALS patients in the late stages of illness and with invasive mechanical ventilation along with their caregivers. ALS patients were assessed with the Hospital Anxiety and Depression Scale, for the evaluation of anxiety and depression, and with the Big Five Questionnaire, a widely used questionnaire about personality traits. Caregivers underwent a semi-structured interview about the patient's personality changes and behaviors.

Results: From the primary analysis conducted personality trait seem to increase after tracheostomy, in particular patients seem to be more obsessive than before the intervention. Qualitative investigation shows that obsessive attitude of patients might affect caregivers well-being and care-burden.

Discussion and conclusion: The first data confirm our hypotheses, from quantitative analysis we might obtain further confirmation and a complete personality profile of ALS patients in late stages. The personality of ALS patients today is not investigated enough, our results could be the basis for further studies. The results will be entirely presented during the symposium.

C68 THE RANGE AND CLINICAL IMPACT OF COGNITIVE IMPAIRMENT IN FRENCH PATIENTS WITH ALS: A CROSS-SECTIONAL STUDY OF NEUROPSYCHOLOGICAL TEST PERFORMANCE

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Keywords: cognitive impairment, frontotemporal dementia, neuropsychological assessment

Background: Amyotrophic lateral sclerosis (ALS) and fronto-temporal dementia (FTD) overlap clinically and pathologically in some patients. There appears to be a range of cognitive changes that occur in ALS, from mild frontal syndromes to overt dementia. The prevalence of impairment in French-speaking patients with ALS has not been examined previously.

Objective: To assess the spectrum and clinical associations of cognitive impairment in French patients with ALS, and determine the effect of cognitive impairment on survival in this population.

Methods: One hundred and thirty-one patients were enrolled in a cross-sectional cohort study of neuropsychological test performance The test battery consisted of measures assessing the following domains: memory (Grober and Buschke verbal episodic memory test; Digit Span and Rey figure recall); language (DO80 naming test); executive function (Stroop test, GoNoGo test; Trail Making Test; verbal fluency; similarities (WAIS III and Wisconsin Card Sorting Test); visual construction (Rey Figure).

ANOVA and chi-square tests assessed differences in clinical characteristics between impaired and unimpaired patients; multiple regression determined which features contributed most strongly to cognitive status; and Cox models compared survival.

Results: Fifty-three patients (40%) were categorized as cognitively impaired based on test performance. Thirteen (10%) patients had frontotemporal dementia (FTD) clinically; all scored in the moderate to severely impaired range on testing. Impaired patients had less education ($p = 0.001$), and severely impaired patients were more likely to have bulbar-onset than unimpaired patients ($p < 0.001$). Severe cognitive impairment predicted shorter survival ($p = 0.007$), even when controlled for motor severity ($p = 0.001$).

Conclusions: Ten percent of a consecutive series of French ALS patients had overt dementia and 40% were cognitively impaired by neuropsychological testing. Lower education level and possibly bulbar-ons*et al*S were associated with impairment. As in other causes of dementia, higher educational attainment may protect against clinical cognitive deterioration in ALS. French patients with severe cognitive impairment have shorter survival time.

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SESSION 9C NEUROIMAGING

C69 THE PAST, PRESENT AND FUTURE OF NEUROIMAGING IN ALS/MND

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Keywords: magnetic resonance imaging, positron emission tomography, biomarker

History may judge the development of neuroimaging as transformational in clinical neuroscience, bringing the post-mortem neuropathological insights of pioneers like Lockhart Clarke and Charcot to the *in vivo* domain. Whilst MRI retains an important role in the exclusion of alternative pathology, advanced applications now provide a parallel role as a source of diagnostic and potentially monitoring biomarkers in ALS (1). In addition, neuroimaging continues to provide important clues to pathogenic mechanisms:

1. Positron emission tomography (PET) studies of cerebral blood flow and metabolism pioneered the concept of ALS as a multiple system disorder (2).
2. Ligand PET revealed cerebral microglial activation *in vivo* (3), underpinning *in vitro* data supporting the pathogenic theme of neuroinflammation in ALS (4). Emerging rodent MRI studies mark an era of molecular imaging, with 'smart' contrast agents targeting surrogate markers of neuroinflammation that may offer therapeutic strategies in human ALS.
3. The marked and similar regional reductions of serotonin (5-HT) 1A receptor binding seen in PET studies of non-demented ALS patients (5) and pure frontotemporal dementia (6) may warrant reappraisal in light of the subsequent emergence of a unifying histopathological marker, namely TDP-43.
4. Flumazenil PET as a surrogate for loss of GABA-ergic function supports the concept of ALS as fundamentally involving a 'failure of cortical inhibition' (7), and so possibly as an 'interneuronopathy' by implication. Developments in computational neuroscience and biostatistics mean that combined structural and functional cerebral connectivity can now be studied non-invasively using MRI. With the addition of high-field magnetic resonance spectroscopy, there is potential to test the hypothesis of imbalance between inhibitory and excitatory neuronal activity in ALS, with potential for improved therapeutic strategies. Connectivity studies in pre-symptomatic carriers of ALS-related gene mutations might reveal an inherently vulnerable motor network, with implications for identifying the larger at-risk population that will be essential to any longer-term aspiration for primary prevention of the sporadic disorder.

MRI is a largely non-invasive, ubiquitous but expensive technology. Whether it can deliver, at routine clinical scanner field strengths, on the promise of a biomarker sensitive enough in the context of a therapeutic trial is being tested through emerging international collaboration (8). MRI may ultimately be only one part of a multimodal biomarker panel that includes biofluid and neurophysiological measurements. However it looks certain to continue to make important contributions to understanding *in vivo* disease mechanisms.

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C70 MAGNETIC RESONANCE MICROIMAGING OF THE SPINAL CORD IN THE SOD1 MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS DETECTS MOTOR NERVE ROOT DEGENERATION

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Keywords: MRI, SOD1 mouse, spinal cord imaging

Current imaging studies in ALS have concentrated on areas of the brain and spinal cord that contain mixed populations of sensory and motor neurons. In this study, *ex vivo* magnetic resonance microimaging (MRM) was used to separate motor and sensory components by visualizing individual dorsal and ventral roots in fixed spinal cords. MRM at 15 μm in plane resolution enabled the axons of pure populations of sensory and motor neurons to be measured in the lumbar region of the SOD1 mouse model of ALS. MRM signal intensity increased by 38.3% ($p < 0.05$) exclusively in the ventral motor nerve roots of the lumbar spinal cord of ALS-affected SOD1 mice compared to wildtype littermates. The hyperintensity was therefore limited to white matter tracts arising from the motor neurons, whereas sensory white matter fibres were unchanged. Significant decreases in ventral nerve root volume were also detected in the SOD1 mice, which correlated with the axonal degeneration observed by microscopy. These results demonstrate the usefulness of MRM in visualising the ultrastructure of the mouse spinal cord. The detailed 3D anatomy allowed the processes of pure populations of sensory and motor neurons to be compared. This has been the first study to use MRM in the mouse spinal cord to detect nerve root volume loss. In the future, these MRM techniques may translate to the study of human ALS patients.

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C71 MRI EVIDENCE OF DISEASE IN PRE-SYMPTOMATIC SOD1 + INDIVIDUALS AT RISK FOR DEVELOPING FAMILIAL ALS

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Keywords: MRS, DTI, pre-symptomatic

Background: It has been speculated that amyotrophic lateral sclerosis (ALS) is characterized by a pre-manifest period during which neurodegeneration precedes the appearance of clinical manifestations. Evidence to support this hypothesis derives primarily from the SOD1 mouse model of ALS but also from preliminary data in a small number of healthy individuals carrying a mutation in the SOD1 gene and at risk for developing ALS.

Methods: The Pre-familial ALS (Pre-fALS) study is an ongoing prospective observational study of people who are at risk for developing familial ALS; participants are recruited from amongst healthy relatives of patients with fALS due to mutations in known susceptibility genes such as SOD1, TDP43 and FUS. Study participants are seen at least annually and undergo multi-modal (clinical, electrophysiological, imaging and "wet") biomarker studies. All pre-symptomatic subjects have no clinical manifestations of disease, normal forced vital capacity, and normal electromyographic examination. Age-matched, healthy gene negative subjects and patients with ALS (either familial or sporadic) were recruited as "comparison" populations. Diffusion tensor imaging of the brain and cervical spinal cord, as well as magnetic resonance spectroscopy (MRS) of the cervical spinal cord, were performed as described elsewhere (1,2).

Results: DTI data are currently being analyzed. Here we present the results of ^{1}H-MRS of the cervical spine performed on 29 healthy controls, 24 pre-symptomatic SOD1 + volunteers, and 23 ALS patients. Compared to controls, NAA/Cr and NAA/Myo ratios are reduced in both SOD1 + subjects (39.7%, $p = 0.001$ and 18.0%, $p = 0.02$) and ALS patients (41.2%, $p < 0.001$ and 24.0%, $p = 0.01$). Myo/Cr is reduced (10.3%, $p = 0.02$) in SOD1 + subjects compared to controls, but no difference was observed between ALS and controls. By contrast, compared to controls, NAA/Cho is reduced in ALS patients (24.0%, $p = 0.002$), but not in pre-symptomatic SOD1 + subjects.

Conclusions: Changes in neurometabolite ratios in the cervical spinal cord (i.e. reductions in NAA/Cr and NAA/Myo) are evident in pre-symptomatic SOD1 + people in advance of symptoms as well as clinical and electromyographic signs of disease. These cross-sectional neurometabolic findings resemble those observed in patients with clinically apparent ALS; they suggest that neurometabolic changes occur early in the course of the disease process. These findings await confirmation from longitudinal imaging data collected as part of the Pre-fALS study.

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C72 *IN VIVO* NEUROPATHOLOGY OF ALS: MULTIMODAL MRI REVEALS EXTENSIVE CENTRAL AND FRONTOTEMPORAL WHITE MATTER DAMAGE

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Keywords: multimodal MRI, diagnosis, biomarker

Background: Visualizing the subtle neuron damage of ALS in MRI has recently been facilitated by computerized statistical image analysis methods. Voxel-based morphometry (VBM), diffusion tensor imaging (DTI) and resting state functional MRI (rsfMRI) have reported consistent changes in ALS; data on magnetization transfer imaging (MTI) and susceptibility weighted imaging (SWI) are controversial. Yet few multimodal MRI data are available which enable neuropathological *in vivo* studies using the different sequences to display several ALS related damage mechanisms simultaneously.

Objectives: To extract typical patterns of ALS damage in VBM, DTI, MTI, rsfMRI and SWI in a group of well characterized ALS patients without manifest dementia; to identify areas of concurrent or alternative MRI damage signature; and to define a set of ALS signature areas for future MRI diagnosis of ALS.

Methods: We investigated ALS related brain damage in 38 ALS patients (age 62.5 + -10.1 yrs, 23 male) with an average ALSFRS-R of 36.8 + -6.9, and 37 healthy, age and sex matched volunteers, using a Siemens Sonata 1.5T scanner. For every patient and volunteer MMSE, FAB, EQ-5D and SF36 scores were obtained. We used MATLAB R2009b (TheMathworks,Natick,USA) as mathematical framework, SPM8 (Wellcome Trust Centre for Neuroimaging, UCL, London, UK), the VBM8 toolbox (Christian Gaser), and FSL/Freesurfer software for preprocessing raw data and assessing results. For white matter analysis we altered the classical VBM approach to spatially normalized, bias corrected T1-contrasted images for SPM-like group comparisons.

Results: Using VBM on grey matter, volume reduction was seen in central areas and frontal areas concurrent with known changes. White matter changes, however, were extensive in custom-masked datasets with a typical frontotemporal distribution, along the subcentral matter and the corticospinal tracts, which was confirmed in diffusion based datasets. These displayed different areas of damage in CST, subcortical central and frontal white matter depending on the DTI parameter analyzed, and may differentiate between early, partly functional, and late, structural damage. RsfMRI confirmed alterations of in particular the motor resting state network. SWI and MRI confirmed substructural damage in the same areas as T1 white matter images indicating macromolecular alterations.

Discussion and conclusion: VBM still produces inconstant results in the grey matter of ALS patients, possibly due to large interindividual variability. In contrast, white matter space is less variable between subjects, and ALS typical changes were seen in a large array of MRI sequences which

may in part display different degrees of damage, and possibly different pathophysiological processes. Consistent changes across sequences were seen in the corticospinal tract, the central corpus callosum and the frontal white matter. These should be used as landmarks for ALS single subject multimodal MRI which in the future should provide valid diagnostic and biomarker information.

C73 A LONGITUDINAL 4 TESLA MRI STUDY OF THE ANTERIOR CINGULATE

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Keywords: anterior cingulate, fluency, MRI

Background: Abnormalities in the anterior cingulate have been identified in multiple ALS studies (1,2) and correlate with cognitive deficits. A recent 4 Tesla study (3) in non-demented ALS patients also found a correlation between anterior cingulate abnormalities and behavioral signs of apathy. No ALS imaging studies, however, have attempted to measure these changes longitudinally to determine how or when they develop.

Objectives: To investigate longitudinal MRI changes, focusing on the anterior cingulate cortex as the primary region of interest (ROI).

Methods: We analyzed longitudinal scans from seventeen ALS patients. Baseline and follow-up T1-weighted images and DTI were scanned at 4.0-Tesla (Bruker/Siemens). Grey matter volume and white matter FA were measured in similar anatomical regions (ROI), and annual change and change rates were calculated. Differences between baseline and follow-up measurements were assessed using a paired-sample t-test and a linear mixed-effects model, with significance set at $p < 0.05$. Apathy was assessed at both time points using the Frontal Systems Behaviour Scale (FrSBe) and verbal fluency, a focused measure of cognition, was assessed using the Delis Kaplan Executive Functions System (DKEFS).

Results: The mean age of the cohort was 59 years with mean duration between scans of 7.4 months (range 5-11).Significant FA changes were noted in multiple anterior cingulate regions using DTI (most significant left caudal anterior cingulate, $p = 0.003$, paired t-test).Using a linear mixed-effect model that controls for variance in age and scan duration, we found gray matter volume changes in the left caudal anterior cingulate which trended towards significance ($p = 0.09$) but no significant DTI findings remained. No significant declines in apathy were noted between the time points, and practice effects were suggested for phonemic fluency. When contrasting semantic fluency (Category Fluency) scores with semantic set shifting (Category Switching) scores, we observed a non-significant decline over time. Correlation between this fluency contrast score and FA values in the most anterior section of the right cingulate gyrus trended towards significance ($p = 0.061$).

Discussion and conclusions: Subtle MRI changes in the anterior cingulate may occur in non-demented ALS patients over a relatively short time period, even though significant clinical changes were not seen and would not be expected during this time frame. These longitudinal findings lend information to earlier work where we identified abnormalities in the right anterior cingulate at baseline. In the current analysis, anterior cingulate changes were primarily left-sided. Future studies require a control group for deeper comparisons.

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SESSION 10A TARGET PATHWAYS AND THERAPEUTIC STRATEGIES

C74 MASS SPECTROMETRY BASED PROTEOMIC ANALYSIS OF CSF FOR BIOMARKERS OF ALS

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Keywords: proteomics, mass spectrometry, biomarkers

Background: There have been numerous studies reporting protein-based biomarkers in the cerebrospinal fluid (CSF) of ALS patients. Most studies have focused on candidate biomarkers based on biochemical pathways presumably involved in ALS. We previously performed an unbiased screen using mass spectrometry based proteomics to discover candidate biomarkers for ALS. To further our understanding of the pathogenic mechanisms of ALS and verify biomarker candidates, we performed a large unbiased proteomic analysis of CSF from ALS and control subjects.

Objectives: Perform an unbiased proteomic analysis of CSF from ALS, healthy control and disease controls using liquid chromatography tandem mass spectrometry (LC-MS/MS) to sequence the proteome in each subject group. CSF from 250 subjects was used for this study.

Methods: CSF was collected and immediately processed from 90 sporadic ALS, 20 familial ALS, 80 healthy control, 20 multiple sclerosis (MS), 20 Alzheimer's disease (AD), 10 upper motor neuron disease and 10 lower motor neuron disease subjects. We created 25-pooled samples for further analysis, each containing 10 subjects of the same gender ratio (6 male, 4 female) and were controlled for age. For ALS samples, each pool was also controlled for site of disease onset, sporadic or familial disease, and use of riluzole. Samples were carefully controlled for removal of abundant proteins, trypsin digestion, enrichment of peptides and analysis by LC-MS/MS using a Thermo LTQ orbitrap mass spectrometer. Proteins were identified by the presence of at least 2 peptides.

Results: Over 4,000 proteins were identified in the human CSF proteome, with significant alterations in 187 proteins between ALS and control groups. Unbiased cluster analysis easily distinguished ALS from the other subject groups. We identified alterations in CSF proteins for a number of signaling pathways and proteins expressed by neurons and/or glia. Specific pathways that exhibited a number of protein alterations in the CSF of ALS patients include the ubiquitin degradation system, RNA/DNA binding proteins, inflammatory proteins, extracellular matrix, cytoskeletal proteins and complement proteins. We also noted age-related changes in the proteome and differences based on site of disease onset.

Discussion: We performed an unbiased proteomic analysis of CSF from ALS and control subjects using LC-MS/MS to identify as many proteins as possible across all subject groups. While we verified findings for many proteins, we also identified additional proteins that are altered in the CSF of ALS patients and mapped these to specific biochemical pathways and expression by cell type. Further studies are required to validate these findings in another separate set of samples collected in a prospective manner across multiple clinics.

Conclusions: We have identified additional candidate biomarkers for ALS and have shown that LC-MS/MS based peptide analysis can distinguish ALS from neurologic disease controls, disease mimics and healthy control subjects.

C75 CHEMICAL GENETIC SCREENS FOR *IN VIVO* TDP-43 MODIFIERS AND ALS DRUG DISCOVERY

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Keywords: TDP-43, FUS, zebrafish, C.elegans

Background: Although our understanding of the genetics and molecular mechanisms leading to ALS and other motor neuron diseases has increased, there is no effective treatment for these disorders. Further, no high-throughput chemical screens have been thus far conducted to identify potential therapies using *in vivo* models for ALS and other motor neuron diseases.

Objectives: Our laboratories have developed stable transgenic lines expressing mutant and WT human TDP-43 and FUS in zebrafish (*D. rerio*) and worms (*C. elegans*). These models are invaluable to study genetic pathways of disease. Further, both these model organisms are amenable to chemical screening.

Methods: We established mutant and WT TDP-43 and FUS lines under an inducible ubiquitous heat shock promoter (zebrafish) or a constitutive motor neuron promoter (*C. elegans*). Mutant but not WT TDP-43 and FUS fish and worms had visible motor deficits accompanied with axonal abnormalities and protein aggregates in motor neurons. Zebrafish larvae and adult worms were placed in multiwall plates containing a variety of chemical compounds. Following overnight treatment, the motor phenotype was assessed. Further, axonal projections from motor neurons as well as protein aggregation using specific antibodies were quantified.

Results: In a small preliminary screen of compounds with neuroprotective properties, we identified Methylene Blue (MB) to consistently and potently rescue the motor phenotype in both our model organisms. Using the power of worm

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genetics, we further studied the chaperones in a variety of subcellular compartments upregulated by MB. We identified that treatment with MB specifically and solely upregulated one protein chaperone. Further, molecular analysis demonstrated that overexpression of mutant TDP-43 elicited cellular stress and specific inhibition of this cellular stress rescued the motor phenotype observed both in worms and zebrafish. Further, MB as well as reducing cellular stress were able to reduce motor neuron excitoxicity by glutamate treatment in mammalian primary motor neuron cultures.

Discussion: We have identified MB as a novel compound with therapeutic potential in ALS. MB rescued the motor phenotype induced by overexpression of both mutant TDP-43 and FUS. Since MB is a compound that is also under clinical trial in Alzheimer patients, this treatment might have significant effects in a number of neurological disorders. We have further elucidated the molecular mechanism through which MB exerts its potential beneficial effect to reduce protein aggregation by upregulating a specific chaperones and reducing cellular stress. Thus, our observations are consistent with other studies suggesting that reduced cellular stress is a crucial step in ALS pathogenesis.

Conclusions: This is the first chemical screen in multiple model organisms for TDP-43 and FUS. Its high-throughput potential and the confirmation of therapeutic compounds in different models give our assay unique capabilities in the quest to treat ALS and other motor neuron diseases.

C76 RESCUE OF MOTOR NEURON DEATH BY TARGETING THE BNIP3 PATHWAY

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Keywords: BNIP3, mitochondria, cell death pathway

Oxidative stress, mitochondrial dysfunction and morphologically necrotic-like motor neuron death are major features in ALS. Previously we showed that oxidative stress provided a redox signal to activate hypoxia-inducible factor 1a (HIF-1a), which is the primary, if not the only, transcriptional factor for the death-inducing gene BNIP3.Expression of BNIP3 caused a caspase-independent form of neuronal cell death *in vitro* and *in vivo*. Here we show that BNIP3 was induced to express at the onset of the disease in transgenic mice expressing the G93A and the G37R mutations of SOD1. BNIP3 was not detectable in the brain of control animals and in the G93A and the G37R mice before the onset of disease. Levels of BNIP3 expression increased with disease progression as evidenced by immunohistochemistry, Western blotting and RT-PCR analyses. The expressed BNIP3 was found to be primarily localized in motor neurons. BNIP3 was not detectable in the liver, kidney and lung tissues from the same groups of G93A and G37R animals that showed high levels of BNIP3 in the spinal cord.BNIP3 was detected in the mitochondrial membranes after alkaline extract, indicating that the expressed BNIP3 was active because inactive BNIP3 is known to be dissociated from mitochondria after alkaline treatment. To further determine the role of BNIP3 in mutant SOD1-induced neuronal death, a lentiviral shRNA vector targeting the nucleotides 167-188 of the BNIP3 mRNA, was injected into the lumbar spinal cord of the G93A mice at the age of 8 weeks. Animals injected with a scramble shRNA vector were used as controls. Inhibition of BNIP3 by RNAi significantly increased the number of axons in the L5 ventral roots ($p = 0.015$). Analysis of axon size distribution showed clearly the protection of middle to large (larger than 6 mm in inner diameter) axons by the lentiviral BNIP3 shRNA vector. We further analyzed the BNIP3 pathway and found that BNIP3 interacted with the ion channel VDAC to induce mitochondrial release of endonuclease G leading to a caspase-independent apoptosis. To look for an inhibitor for the BNIP3 pathway, we identified the small chemical necrostatin-1 that was able to inhibit BNIP3 cell death pathway by preventing integration of BNIP3 to the outer membrane of mitochondria. The results demonstrate that BNIP3 plays a role in mediating mutant SOD1-induced motor neuron death. The BNIP3-induced cell death pathway provides a molecular linkage for mitochondrial degeneration, oxidative stress and caspase-independent neuronal death. Necrostatin-1 appears to be a potent inhibitor for the BNIP3 pathway and may be a new therapy for ALS.

C77 THE BIS(THIOSEMICARBAZONATO)-COPPER^{II} COMPOUND CU^{II}(atsm) DELAYS SYMPTOM ONSET AND DISEASE END-STAGE IN SOD1G37R MICE

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Keywords: therapeutic, mouse models, SOD1

Background: In the absence of effective treatments for ALS the development of new therapeutic strategies is still needed. In preliminary pre-clinical studies we tested the *bis* (thiosemicarbazonato)-copper^{II} compound Cu^{II}(atsm) as a novel therapeutic for ALS. Treating mice expressing a low copy number of human SOD1G93A slowed progression of disease symptoms and significantly delayed the age at which the mice reached disease end-stage.

Objectives: In the present study we tested therapeutic activity of Cu^{II}(atsm) in mice expressing human SOD1G37R.Our objective was to determine whether Cu^{II}(atsm) could prevent disease symptoms in a second, more aggressive mouse model for ALS.

Methods: Mice expressing human SOD1G37R and their non-transgenic littermates were treated with Cu^{II}(atsm) at 30 mg/kg body weight (or with the suspension vehicle as a sham control) by daily gavage. Cu^{II}(atsm) is not commercially available and was synthesised following published procedures. The onset and progression of locomotor deficits were monitored using rotarod and stride length assays. One cohort of mice was monitored until they reached disease end-stage and a second cohort of mice was culled at 24 weeks when disease symptoms became apparent in the sham treated mice. Tissues were collected from culled mice for immunohistochemistry and biochemical analyses.

Results: Treating with Cu^{II}(atsm) significantly delayed the onset of disease symptoms in SOD1G37R mice. Sham treated SOD1G37R mice developed an impairment on the rotarod at 22.5 weeks of age and in the stride length assay at 24 weeks. These deficits however were not detectable in Cu^{II}(atsm) treated mice until 26.5 weeks (rotarod) and 28 weeks (stride length assay).Irrespective of when disease symptoms developed Cu^{II}(atsm) did not alter the rate of their progression.

Accordingly, disease end-stage was delayed in the Cu^{II}(atsm) treated mice by 4 weeks. Western blot analysis of whole blood samples collected from mice at 24 weeks revealed treating with Cu^{II}(atsm) did not alter expression of the endogenous mouse SOD1. By contrast, levels of the transgenic human SOD1G37R were increased 2.3-fold in the Cu^{II}(atsm) treated mice.

Discussion and conclusions: These data support our previous work using low copy SOD1G93A mice and demonstrate that Cu^{II}(atsm) delays the onset of disease symptoms in the more aggressive SOD1G37R mouse model for ALS. Increased levels of SOD1G37R in the blood of Cu^{II}(atsm) treated mice indicate that stabilisation of the mutant SOD1 may contribute to the therapeutic activity of Cu^{II}(atsm). Our on-going histological and biochemical analyses of brain, spinal cord and muscle samples should reveal more information on the mechanism of action for this potential ALS therapeutic.

C78 INTERMOLECULAR AND INTERCELLULAR PROPAGATION OF SUPEROXIDE DISMUTASE 1 MISFOLDING IN ALS

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Keywords: superoxide dismutase 1, prion, template-directed misfolding

Background: Approximately 10% of ALS cases are familial, with ~20% of these due to mutations in the gene encoding Cu/Zn superoxide dismutase 1 (SOD1), a widely-expressed free-radical defense enzyme. A consequence of SOD1 mutation and/or oxidation is a propensity of the protein to misfold and aggregate. Human wild-type (wt) SOD1 is known to co-aggregate with mutant SOD1 in familial ALS, in double transgenic mouse models, and in cell culture systems, but the structural determinants of this process and its functional consequences are unclear.

Objective: We sought to molecularly dissect the effects of intracellular obligately misfolded SOD1 mutant proteins on natively structured wild-type SOD1.

Methods and results: Expression of the enzymatically inactive, natural familial ALS SOD1 mutations G127X and G85R in human mesenchymal and neural cell lines induces misfolding of wild-type natively-structured SOD1, as indicated by: 1) acquisition of immunoreactivity with SOD1 misfolding-specific monoclonal antibodies; 2) markedly enhanced protease sensitivity suggestive of structural loosening; 3) non native disulfide-linked oligomer and multimer formation; and 4) generation of reactive oxygen species. Expression of G127X and G85R in mouse cell lines did not induce misfolding of murine wtSOD1, and a species restriction element for human wtSOD1 conversion was mapped to a region of sequence divergence in loop II and beta-strand 3 of the SOD1 beta-barrel (residues 24-36), then further refined surprisingly to a single tryptophan residue at codon 32 in human SOD1. Aggregated recombinant G127X is capable of inducing misfolding of recombinant human wtSOD1 in a cell-free system in buffered saline containing reducing and chelating agents. The presence of a tryptophan at codon 32 on recombinant G127X increases its effectiveness at inducing wtSOD1 misfolding, compared to a serine at position 32 as found in the murine SOD1 sequence. Furthermore, the culture medium from cells transiently transfected with wild-type or mutant SOD1 can induce misfolding of endogenous SOD1 when added to naive neuroblastoma cell cultures, and this process can be stably propagated in serial passage. The agent responsible for induction of misfolding was determined to be a misfolded SOD1 aggregate. Transmission of SOD1 misfolding *in vitro* is abrogated by extracellular pan- and misfolding-specific SOD1 antibodies.

Conclusions: SOD1 misfolding and toxicity can propagate within and between cells, which may prompt novel targeted therapies for ALS and other neurodegenerative diseases.

C79 NON-VIRAL GENE DELIVERY FOR MOTOR NEURONS

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Keywords: non-viral gene delivery, SOD1G93A mice, antibodies

Background: There are no effective treatments for motor neuron disease (MND) also known as Amyotrophic Lateral Sclerosis (ALS). We have developed a non-viral method of introducing exogenous genes into neurons using antibodies to specific receptors as targeting agents, which are termed 'immunogenes'. The common neurotrophin receptor (p75NTR) is up-regulated on motor neurons of symptomatic SOD1G93A mice, the mouse model of MND (1) whereas the trkC receptor is present on motor neurons throughout the entire lifespan (2). Our group has shown functional outcomes are possible by specific delivery of siRNA to basal forebrain neurons, and GDNF encoding plasmid to injured motor neurons using immunogenes targeting the p75NTR (3,4).

Objectives: To demonstrate that immunogenes targeting either p75NTR or the TrkC receptor can deliver genes to motor neurons of SOD1G93A mice.

Methods: p75NTR and trkC targeting antibodies MLR2 and 2B7 were tested for ability to access motor neurons following systemic injections into mice *in-vivo*. Immunogenes were constructed by attaching polyethylene glycol (PEG) covalently to the DNA/ RNA binder polyethylenimine (PEI) that was then conjugated to monoclonal antibodies. The specificity and toxicity of the immunogenes were examined in primary motor neuron cultures. The ability of p75NTR immunogenes to target motor neurons in SOD1G93A mice was examined by intraperitoneal injections of immunogenes carrying plasmids that express green fluorescent protein (pGFP).

Results: Administration of p75NTR antibody by systemic injections into newborn mice (n = 4), showed that the antibody internalises into more 90% of motor neurons. TrkC receptor antibody also was able to access adult motor neurons after intraperitoneal injections (n = 3). PEG was attached covalently to PEI and then conjugated to the monoclonal antibodies. Immunogenes condensed and bound plasmid encoding GFP (n = 3). MLR2-PEG-PEI-pGFP was able to transfect pure motor neurons cultured in the presence of 10% serum (n = 4; 5) and motor neurons in mixed cultures containing primarily astrocytes (n = 3). In addition, MLR2-PEG-PEI-pGFP was

not toxic to cultured neurons (n = 4). Finally, control mice given 2B7-PEG-PEI-pGFP or symptomatic SOD1G93A mice given intraperitoneal MLR2-PEI-PEG-pGFP resulted in GFP expression in spinal motor neurons (n = 4).

Discussion and conclusion: This study shows effective non-viral gene delivery to motor neurons *in-vitro* and *in-vivo*. Reduced toxicity and stability of the immunoporter may be important in developing gene therapies that target motor neurons in the SOD1G93A mice.

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SESSION 10B CLINICAL TRIALS

C80 ALS TRIAL DESIGN: CAN WE DO BETTER?

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Keywords: clinical trials, trial design, historical controls

The data from Phase II trials are critical to informing the decision about moving forward into Phase III. Phase II methods are particularly important in ALS, where the opportunity to test many promising agents must be balanced against relatively low funding and a paucity of patients enrolling in trials. Innovative approaches in recent trials provide important clues for improving efficiency of ALS trials.

The goals of Phase II trials are to examine a biologic effect, evaluate dose ranging and pharmacokinetics, and look for a hint of efficacy. The biologic effect is best shown through an impact on a biomarker. An example is the beneficial change in a biomarker of macrophage activation after a single dose of NP001 (Neuraltus) reported elsewhere at this meeting.

Another critical issue is dose finding. The failure of high dose minocycline provides one example of insufficient attention to dose ranging. In contrast, the current phase III trial of Dexpramipexole (Knopp) shows how phase II dose-response data may provide a basis for moving to Phase III.

New ALS trial designs show great promise. Small futility trials can benefit the field by eliminating agents not meeting a pre-set target. The recent NEALS lithium trial, using a novel "6 point drop" endpoint, reduced trial time and excluded the large benefit that had been reported in an earlier trial. The futility study of Coenzyme Q10 used a two-stage design to compare different doses, before testing the "winner" against placebo in stage two.

Historical placebo controls aim to further reduce costs and increase power, and are attractive to subjects who are more likely to receive drug. Critical to this method is excluding any disease-related drift of endpoints over time, which occurred in Parkinson's disease. Our placebo database, of 475 controls from six ALS trials, shows no change in the rate of functional decline over the past decade, provided that patients are matched for differences in inclusion criteria. This provided a basis for a WALS historically controlled lithium trial, which reached conclusions that were similar to lithium studies using contemporary controls (NEALS, Chio 2010). Increased efficiency might result from combining concurrent and historical controls, thereby reducing sample size, in terms of new enrollment, required for a predefined power. Shorter duration (6 months) trials using endpoints such as ALSFRS and FVC also reduce cost and sample size compared to trials based on survival.

Renewed focus on the goals and efficiencies of phase II trials in ALS will move the field forward. There is room for improvement also in phase III regarding inclusion criteria, endpoints and trial design.

C81 LITHIUM WITH RILUZOLE FOR THE TREATMENT OF ALS: A RANDOMISED, SEQUENTIAL, PLACEBO-CONTROLLED TRIAL ON SURVIVAL

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Keywords: lithium, trial, survival

Background: We studied the effects on survival of lithium in combination with riluzole in patients with ALS. After the publication of a pilot study in 2008 reporting lithium could slow disease progression in ALS, a number of clinical trials were set up to examine its true effects. Thus far, several of these trials have published their results, none of these were able to reproduce the positive findings of the initial pilot study. However, the effect of lithium on survival in patients with ALS remained unknown.

Objectives: To study the effects of lithium versus placebo on survival in ALS with a randomised, sequential trial.

Methods: A randomized, sequential trial with lithium versus placebo was performed in 133 patients with ALS. Between November 2008 and June 2011, patients were randomized to receive lithium carbonate (target blood level 0.4 - 0.6) or placebo. Primary end point was survival. Secondary outcome measures were decline of functional status measured by the revised ALS Functional Rating Scale and vital capacity. Analysis was by intention to treat and according to a sequential trial design. Secondary outcome measures were analysed using a linear mixed-effects model. This trial was registered with the Netherlands trial registry (number NTR1448).

Results: A total of 61 patients reached a primary endpoint, 33 of 66 in the lithium group compared with 28 of 67 patients in the placebo group. Lithium did not significantly affect survival (cumulative survival probability of 0.73 in the lithium group (standard error (SE), 0.06) versus 0.75 in the placebo group (SE 0.06) at 12 months and 0.62 in the lithium group [SE 0.06] versus 0.67 in the placebo group (SE 0.06) at 16 months). In addition, the rate of functional decline did not differ between treatment groups ($p = 0.97$). There were no major safety concerns.

Conclusions: We found no evidence that lithium in combination with riluzole affects survival or functional decline of patients with ALS. The current study is the first report of a placebo controlled trial with lithium taking survival as primary endpoint. In addition, the trial was designed to detect small beneficial treatment effects similar to that of riluzole. However, with the cumulative evidence currently available, we unfortunately conclude lithium has not shown to benefit patients with ALS.

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C82 A RANDOMIZED, DOUBLE-BLIND, DOSE-RANGING STUDY OF MEMANTINE IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: randomized controlled trial, memantine, human

Background: Toxicity caused by glutamate contributes to motoneuron (MN) destruction in ALS. While memantine inhibits the deleterious effects of glutamate, it does not affect its normal action and has been shown to prolong survival in SOD1 transgenic mice.

Objectives: 1) to test the hypotheses that memantine would slow MN degeneration and functional decline; 2) to identify the optimum dose.

Methods: Adult ALS subjects with symptom duration < 3 years and forced vital capacity (FVC) $> 60\%$ predicted were recruited. Motor unit number estimation (MUNE) was done on the median innervated hand muscles using multipoint stimulation. To gauge upper motoneuron (UMN) changes, n-acetylaspartate levels in the motor cortex were measured using magnetic resonance spectroscopic imaging (MRSI). Functional changes were evaluated using the ALSFRS-R, FVC and manual muscle testing (MMT). At the end of a 4 month run-in period, subjects were randomized to either receive low dose (5 mg bid) or high dose (10 mg bid) memantine for 5 months. MUNE and MRSI were done at baseline and repeated at the end of the run-in and treatment phases. Functional measures were checked monthly throughout the study. Double blinding was maintained by a drug safety monitoring board that worked at arms length and was responsible for randomization, data analysis and monitoring of adverse events. Medication compliance was monitored through monthly serum samples.

Results: Of 29 potentially suitable subjects screened, 24 who qualified were equally divided to each treatment arm. One patient in the high dose group died leaving 11 subjects available for final analysis while in the low dose group, 2 patients died and 1 withdrew leaving 9 subjects. Serum concentration analysis revealed good compliance. Reported adverse events were similar between the run-in and treatment phases. There was significant slowing of spinal MN loss in the high dose group from -12.4 ± 3.7 (mean $\pm$ sd)/month at run-in to -5.3 ± 2.2/month during treatment ($p = 0.03$). When all subjects were combined, they showed a trend of slowing in spinal MN loss that approached significance (-7.8 ± 2.4/month at run-in vs. -4.2 ± 1.3/month during treatment, $p = 0.05$). However, none of the functional measures or MRSI revealed a significant treatment effect: ALSFRS-R -1.5 ± 0.2 vs. -1.2 ± 0.2, $p = 0.22$; FVC -2.3 ± 0.7 vs. $-2.4 \pm 0.7\%$, $p = 0.96$; MMT -6.6 ± 1.2 vs. -7.1 ± 1.3, $p = 0.69$; MRSI 0.0004 ± 0.002 vs. $+0.0006 \pm 0.004$, $p = 0.86$.

Discussion and conclusions: In this pilot study, we found that memantine at 10 mg bid was effective in slowing spinal MN loss in ALS patients. However, functional measures and UMN degeneration were not significantly improved. The latter could be due to the small sample size. Based on these observations, the fact that memantine is well tolerated and that a previous human study in ALS patients also showed benefits, a multicentre full scale clinical trial aimed at recruiting patients at earlier stages of ALS may be warranted.

C83 A FIRST-TIME-IN-HUMAN STUDY IN ALS PATIENTS WITH THE ANTI-NOGO-A MONOCLONAL ANTIBODY GSK1223249: PRELIMINARY RESULTS

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Keywords: Nogo-A, monoclonal antibody, therapeutic trial

Background: Nogo-A (neurite outgrowth inhibitor A) is a high molecular weight transmembrane protein initially identified as a potent myelin-associated inhibitor of axonal growth expressed mostly by oligodendrocytes. Over-expression of Nogo-A in skeletal muscle of amyotrophic lateral sclerosis (ALS) patients has been implicated in the pathophysiology of this disease.

GSK1223249 is a humanized monoclonal antibody (mAb) that potently binds to a specific epitope on Nogo-A to neutralize/antagonize this protein. It is hypothesized that GSK1223249 may promote neuro-regeneration and synaptic plasticity, thus leading to clinical improvement or slowing of disease-progression in certain degenerative neurological disorders such as ALS.

Objectives: GSK has conducted a First-Time-in-Human trial (FTIH) with this mAb between 2009 and 2011 with the primary objective to assess its safety & tolerability in ALS patients. Secondary objectives included an investigation of its pharmacokinetics (PK), immunogenicity and effects on different clinical and pharmacodynamic (PD) endpoints, such as ALSFRS-R scores, Slow Inspirational Vital Capacity (SVC), Motor Unit Number Estimation (MUNE) and biochemical biomarkers in skeletal muscle tissue.

Methods: The FTIH NOG111330 trial was a randomized, placebo-controlled, double-blind, multi-centre, sequential dose escalation, 2-part fusion protocol. Seventy-six patients with ALS were randomized 3:1 (active: placebo) and enrolled into 8 cohorts, subdivided into 2 parts. In Part 1, single escalating intravenous (i.v.) doses of GSK1223249 were evaluated in 5 sequential patient cohorts (0.01 to 15 mg/kg, n = 8/cohort), whilst in Part 2 patients in each of the 3 cohorts received 2 repeat i.v. doses approximately 4 weeks apart (0.5 to 15 mg/kg per dose, n = 12/cohort).

Results: The trial is currently ongoing, but it will have concluded by the time of the meeting, and headline data on the safety/tolerability, pharmacokinetics and potentially pharmacodynamic endpoints of GSK1223249 in ALS patients will then be available.

Discussion and conclusions: If GSK1223249 is safe and well tolerated with a favourable PK profile and demonstrates target engagement, then progression to a large proof-of-concept study is currently planned.

C84 EFFECTS OF NP001 TREATMENT ON MONOCYTE/MACROPHAGE ACTIVATION IN PATIENTS WITH ALS

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Keywords: monocyte/macrophage activation, biomarker, NP001

Background: Elevated blood levels of abnormally activated monocyte/macrophages (MO) are present in patients with ALS (1). These CD14 + cells have high levels of activation/inflammation marker HLA-DR and a subset express elevation of the activation marker CD16. NP001 is a systemic macrophage activation regulator for treatment of ALS. A recently completed phase 1 single ascending dose safety and biomarker study in 32 ALS patients established the tolerability and safety of single dose administration of NP001. This abstract details the effects of NP001 on MO HLA-DR and CD16 expression as potential biomarkers for disease activity of ALS.

Objectives: 1) To evaluate the potential of blood MO immune parameters as biomarkers in ALS; 2) To determine blood MO biomarker response to single doses of NP001 in ALS.

Methods: This was a double-blind, placebo-controlled single ascending dose, phase 1 study in 32 patients with ALS. NP001 was administered to 24 patients (6 at each of four different doses: 0.2, 0.8, 1.6, and 3.2 mg/kg) and 8 patients received placebo. Flow cytometry was performed to assess expression of blood MO activation/inflammation markers pre- and 24 hours after a single dose of NP001 or placebo in 32 ALS patients.

Results: HLA-DR and CD16 MO expression demonstrated positive responses to NP001 treatment at 24 hours post-dosing. Reduction of MO HLA-DR expression was directly related to the patient's prior disease progression rate as assessed by ALSFRS-R decline per month (DP rate). Post-dosing, significant dose-independent trends in reduction of HLA-DR expression were seen when patients were categorized into three groups: DP rate: < 0.5, ≥ 0.5 but < 1, ≥ 1 ($r^2 = 0.2922$, $p = 0.0075$). NP001 dose-dependently decreased levels of MO CD16 expression ($r^2 = 0.2352$, $p = 0.0129$). Additionally, there was no relationship between MO marker response and concomitant riluzole use.

Importantly, baseline levels of MO activation defined by CD14 co-expression of HLA-DR was directly related to the patient's DP rate ($r = 0.4310$, $p = 0.0138$). A positive correlation was found between levels of CD16 MO expression and DP rate ($r = 0.4499$, $p = 0.0098$). These two parameters were independent of each other and when combined showed a more powerful relationship with DP rate in ALS (Multiple $R = 0.5734$, $p = 0.0031$).

Conclusions: Two MO activation/inflammation markers, HLA-DR and CD16, were independently associated with DP rate in ALS patients, which suggests their potential as biomarkers desperately needed for ALS. Single doses of NP001 reduced levels of both HLA-DR and CD16 MO expression with the degree of reduction dependent on prior DP rate (HLA-DR) and NP001 dose (CD16). Based on the relationship between DP rate and levels of HLA-DR and CD16 expression in ALS, NP001 induced reduction of MO activation/inflammation may slow disease progression.

Reference

1. Zhang R, Gascon R, Miller RG, *et al.* J Neuroimmunol 2005;159:215–24.

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SESSION 11A DISEASE MODELS

C85 OPTIMISING STEM-CELL DERIVED MODELS FOR ALS RESEARCH

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A number of devastating diseases, including amyotrophic lateral sclerosis (ALS), specifically affect the neuromuscular system. Progress in understanding the molecular pathology underlying these conditions has been slow, partly because it has been impossible to access significant quantities of the disease-affected cell, the spinal motor neuron. With recent advances in stem cell and reprogramming biology, we can now produce millions of spinal motor neurons with control and diseased genotypes. Motor neurons made by stem cell and reprogramming approaches possess a molecular signature similar to embryonic motor neurons and are functional both *in vivo* and *in vitro*. These new cellular resources can now be used to design *in vitro* disease models for both mechanistic studies and for the discovery of novel small-molecule therapeutics.

C86 ARE MICE A GOOD MODEL FOR HUMAN ALS?

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Keywords: mouse models

My research program at The Jackson Laboratory focuses on the power of mouse genetics to understand the cellular and molecular mechanisms of neuromuscular disease. In recent years, however, significant questions about the utility of individual genetic models for predicting the efficacy of therapeutic interventions for amyotrophic lateral sclerosis (ALS) have been advanced by both investigators and funding agencies. Some concern is legitimate and limitations need to be understood and addressed so that investigators can properly plan and interpret their results. For example, the high levels of transgenic over-expression required for initiation of disease symptoms (in the case of SOD1) or the sensitivity of mice to TDP43 or FUS manipulations need to be considered when making conclusions. I would argue, however, that some of these limitations are self-inflicted due to attempts at initiating a disease in mice in less than 4-6 months when the human disease can take longer than 50 years to manifest itself in patients. This is compounded by poorly planned and underpowered mouse genetics experiments that often do not consider the effects that genetic background can have on both disease onset and progression. In my own research, we have taken advantage of these differences in genetic background effects to map modifiers of disease. The genetic heterogeneity observed in familial forms of ALS (FALS) and the high proportion of sporadic cases suggest that several genes are likely to be necessary for the survival of motor neurons. The identification of genetic modifiers in a tractable model system like the mouse offers tantalizing hope that specific proteins and biochemical pathways can be identified to serve as targets for rational therapy development. In addition, ES cell-derived motor neurons from these mouse models along with patient-derived iPS cells will allow direct comparisons of cellular pathway defects, allow modifier gene candidates to be efficiently interrogated and can provide a platform for high-throughput screening of compounds that can then be tested for efficacy in an appropriate animal model.

C87 NEURONAL EXPRESSION OF ALS-LINKED TDP-43 IN MICE IS SUFFICIENT TO TRIGGER ADULT-ONSET MOTOR NEURON DISEASE

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Keywords: TDP-43, mouse model, cell-autonomous

Background: Mutations in TDP-43, a DNA/RNA binding protein involved in RNA transcription and splicing cause familial and sporadic ALS. Accompanied with the clearance of nuclear TDP-43, cytoplasmic and intranuclear aggregates containing TDP-43 have been observed in both neurons and glia in cases of ALS. Whether disease arises from a loss of nuclear TDP-43 function and/or gain of a toxic property by cytoplasmic aggregates remains to be established. It is likewise not clear as to whether both cell-autonomous and non-cell autonomous mechanisms contribute to disease linked to TDP-43. To begin to clarify these issues, we employed both gain- and loss-of-function approaches in mouse model systems and showed that TDP-43 participates in pathways critical for motor neuron physiology, including those that regulate the normal distributions of SMN-associated GEMs in the nucleus and mitochondria in the cytoplasm.

Objectives: The fact that neuronal over-expression of wild type or ALS-linked mutant TDP-43 showed early post-natal lethality in mice precludes the opportunity to assess whether accumulation of wild type or mutant TDP-43 in neurons is sufficient to trigger adult-onset motor neuron disease. To test this hypothesis, we focused on developing transgenic mice expressing lower levels of TDP-43 in efforts to obtain a mouse model that exhibits an adult-onset motor neuron disease.

Methods: In order to drive TDP-43 expression in a neuronal specific manner, we used the Thy 1.2 promoter to develop lines of mice expressing either wild type or ALS-linked mutant TDP-43. Lines of mice expressing low levels of TDP-43 were identified by protein blot analysis. Standard behavioral and pathological analysis of TDP-43 transgenic mice and littermate controls were performed.

Results: We have identified mice expressing a lower level of either wild type or mutant TDP-43. These mice are characterized

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clinically by weakness and showed evidence of hindlimb paralysis near end stage disease; pathologically, there is significant loss of ventral horn motor neurons. In contrast to high expressing lines of TDP-43 mice that exhibit a developmental phenotype, we have developed TDP-43 mouse models that faithfully recapitulate both clinical and pathological features of motor neuron disease.

Discussion and conclusions: The fact that expression of wild type TDP-43 causes adult-onset motor neuron disease would suggest the possibility that increase in TDP-43 gene dosage or mechanism whereby TDP-43 is upregulated may contribute to disease in ALS. Neuronal expression of TDP-43 is sufficient to trigger adult-onset motor neuron disease indicating that cell-autonomous mechanisms are a major driving force in initiating disease in TDP-43 linked ALS. How non-cell autonomous mechanisms contribute to disease pathogenesis remains to be explored. Availability of these new TDP-43 mouse models will be useful for further clarifying disease mechanism and testing therapeutic strategies in the future.

C88 OVEREXPRESSION OF HUMAN WILD-TYPE FUS RESULTS IN MOTOR DYSFUNCTION AND DEATH IN HOMOZYGOUS MICE

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Keywords: FUS, transgenic mouse, frontotemporal dementia

Background: Mutations in the gene encoding the RNA binding protein, Fused in Sarcoma (FUS), have been identified as causing 3-5% of familial ALS cases.FUS containing inclusions have been identified in a subset of both ALS and frontotemporal dementia cases without mutations.

Objectives: To examine the effect of wild-type FUS over expression on motor function in the mouse.

Methods: Mice over-expressing HA tagged human wild-type FUS under the control of the mouse PrP promoter were generated.General health status and motor function was assessed longitudinally using a battery of tests including the rotarod and balance beam. Immunohistochemical analysis of the brain and spinal cord was undertaken using an anti-HA antibody to assess FUS expression and inclusion formation. Antibodies to ubiquitin, TDP-43 and GFAP to assess the extent of any pathology.

Results: Homozygous over-expression of FUS resulted in progressive motor dysfunction, with overt symptom onset at approximately 4 weeks of age. Mice developed a severe tremor and limb paralysis, with an average survival time of 10-12 weeks. Compared to both non-transgenic, and heterozygous littermates, animals displayed a significant reduction in weight, along with a significantly impaired performance on the rotarod and balance beam. Because of the early and severe motor dysfunction it was not possible to reliably assess cognitive function. Immunohistochemical comparison of heterozygous and homozygous FUS over-expressing mice, demonstrated a shift in the cellular distribution of the protein from the nucleus to the cytoplasm in the spinal cord of the homozygous animal.

Discussion and conclusions: The onset of progressive, severe motor dysfunction in homozygous mice over-expressing the wild-type human FUS protein suggests that FUS pathology in ALS and FTD may act via a gain of function mechanism. In addition, the correlation between motor dysfunction and the shift in cellular localisation from the nucleus to the cytoplasm in the spinal cord of the homozygous mouse supports the hypothesis that FUS mislocalisation is linked to pathogenesis.

C89 ALS-LIKE SPINAL CORD PATHOLOGY IN TRANSGENIC MICE WITH A MUTATION IN THE VALOSIN-CONTAINING PROTEIN GENE

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Keywords: transgenic, valosin containing protein, TDP-43

Kimonis *et al.* identified a human genetic syndrome, Inclusion Body Myopathy associated with Paget's disease of the bone and frontotemporal dementia (IBMPFD), and subsequently found it to be associated with mutations in the valosin-containing protein (VCP) gene (Watts, Nature Genetics 2004). A knock-in VCP mouse model of IBMPFD (R155H) developed by this group exhibited muscle, bone and brain pathology characteristic of the human disease, including TDP-43 positive inclusions (Badadani, PLoS One. 2010). Recent studies have extended the list of diseases associated with VCP mutations to include ALS (Johnson, Neuron 2010). We have thus undertaken studies of spinal cord pathology in heterozygous R155H mice. Preliminary examinations of 18-24 month old R155H mice show degenerative changes in ventral horn motor neurons (MNs), and increased astrocyte activation. In addition, we find evidence for TDP-43 positive cytosolic inclusions in many damaged MNs. These studies suggest that the R155H VCP mouse may provide a valuable new animal model for ALS, which reproduces key aspects of human disease, including the presence of MN cytosolic aggregates, and pronounced astrocytic as well as MN pathology.

C90 DEVELOPMENT OF *C. ELEGANS* MODELS FOR AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: C. elegans, TDP-43, FUS/TLS

Background: Two recently discovered causative genes for ALS, TDP-43 (TAR DNA Binding Protein 43) and FUS/TLS (Fused in Sarcoma/Translocated in Liposarcoma), are under further investigation regarding their biological roles in neuropathies. Since TDP-43 and FUS are evolutionarily conserved we turned to the model organism *C. elegans* to learn more about their biological functions.

Objectives: The following objectives are being carried out: investigate the biological role of *tdp-1*/TDP-43 and *fust-1*/FUS in order to better understand where those genes function; generate transgenic *C. elegans* models to investigate the consequences of TDP-43 and FUS/TLS mutations in worms by expressing the 2 genes in the motor neurons, and obtain strains useful for screening.

Methods: The worm orthologues of TDP-43 and FUS are *tdp-1* and *fust-1* and we have obtained deletion mutants for each gene. These mutants are being characterized for their contribution to cellular stress resistance and longevity. We also have taken a transgenic approach to study the *in vivo* consequences of TDP-43 and FUS mutations. We engineered strains to express wild type and mutant human TDP-43 or FUS in worm motor neurons.

Results: We performed lifespan and stress response assays and observed that *tdp-1* and *fust-1* have roles in the response to oxidative and osmotic stress. *tdp-1* regulated lifespan and may be linked to the Insulin/IGF pathway.

For our transgenic experiments, the expression of mutant TDP-43 or FUS in worm motor neurons produces robust, adult onset, age-dependent motility defects ultimately leading to paralysis. These phenotypes are useful for genetic and pharmacological suppressor screening. We have conducted a genetic screen and isolated a number of suppressors of mutant TDP-43 toxicity.

Discussion: Under normal conditions *tdp-1*/TDP-43 seems to have a role in the regulation of specific aspects of cellular stress response. Furthermore, in terms of *tdp-1's* biological role we have identified *tdp-1* as a new downstream regulator of a major and central pathway, the Insulin/IGF signaling path. The transgenic toxic gain of function models allowed us to isolate genetic suppressors of motor neuron cell death and will be useful to find compounds that can suppress cell death.

Conclusion: Together these data provide clues to help unravel the mechanism for TDP-43 and FUS toxicity that should also provide leads for early drug discovery.

C91 ZEBRAFISH MODELS OF ALS/MND: DELINEATING THE ROLE OF MUTANT FUS AND TDP-43

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Keywords: zebrafish, FUS, TDP-43

Background: Motor neuronal cytoplasmic aggregations containing Fused in Sarcoma (FUS) or TDP-43 proteins are a pathological hallmark of ALS. FUS and TDP-43 are highly related, predominantly nuclear proteins that share a similar structure and common role of RNA-binding. Interestingly, mutations in each of the genes encoding these proteins, TARDBP and FUS respectively, have been identified in ALS patients, providing compelling evidence of a putative role for these aberrant proteins in the pathogenesis of ALS.

Objective and method: We have created *in vivo* models of ALS in zebrafish, an animal model that is becoming widely regarded for its advantages in both developmental biology and human disease research. By generating transgenic zebrafish lines expressing specific human FUS or TARDBP mutations identified in ALS families, we are able to investigate *in vivo* the role of mutant FUS and TDP-43 in the pathogenesis of ALS at high temporal and spatial resolution.

Results: Specifically, we have examined the development and morphology of primary motor neurons and neuromuscular junctions, patterns of redistribution and aggregation of FUS and TDP-43, formation of associated stress granules and neuronal degeneration profiles. Functionally, we have also characterised the effects of these mutations on motor activity and general survival.

Discussion and conclusion: Indeed, our transgenic zebrafish models recapitulate several key aspects of human ALS and as such, provide crucial insight into the pathogenic mechanisms involved in this largely enigmatic disease. Moreover, these models are a valuable tool that will ultimately be used for high throughput drug screening and development of more effective therapeutics.

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SESSION 11B RESPIRATORY MANAGEMENT

C92 CLINICAL CHARACTERISTICS AND OUTCOMES OF NIV IN ALS

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Keywords: non-invasive ventilation, outcome

In amyotrophic lateral sclerosis (ALS) the involvement of respiratory muscles can occur at different clinical stages, and respiratory failure is the most frequent cause of death (Gil *et al*, 2008), sometimes complicated by the coexistence of bulbar involvement. A correct management thus requires frequent and accurate evaluations of symptoms and serial tests. According to current clinical guidelines (Andersen *et al*, 2007; Miller *et al*, 2009) and a Cochrane systematic review (Radunovic *et al*, 2009), non-invasive ventilation (NIV) is the treatment of choice in the management of respiratory disturbances in ALS. This statement is largely based on the only controlled trial on NIV in ALS, which demonstrated a clear benefit of this treatment, particularly in patients without severe bulbar symptoms.

In recent years NIV has been increasingly used in current practice. According to a population based study (the Piemonte and Valle d'Aosta register for ALS), the frequency of NIV augmented from 15% in the 1995-1999 period to 25% in the 2000-2004 period. This increase, however, was mainly limited to patients followed by tertiary ALS centers. NIV was performed more frequently by young people, married, and of male gender. Social status can also influence the likelihood to undergo NIV, according to differences in national health systems; in fact sometimes NIV is given free, in other cases could be partly or totally charged to patients. Other (negative) aspects that influence provision of NIV services include the lack of knowledge of many neurologists for timely identification of respiratory problems in their patients, and that not enough chest physicians have the appropriate skills for managing respiratory failure in ALS patients.

Median survival time after NIV is in the range of 6 to 12 months both in clinical and in epidemiological studies. Several factors influence the outcome of NIV. These include patients' clinical status (severity of bulbar impairment, lower ALSFRS-R score, poor nutritional status) and respiratory function (lower forced vital capacity) at the time of NIV initiation. Other factors are patients' age and airway mucus accumulation. Both frontotemporal dementia and isolate executive dysfunction negatively influence the acceptance and the compliance to NIV.

Compliance to NIV can be improved if patients are adequately trained, for example with initial acclimatization, possibly in a hospital multidisciplinary setting with specific competences; careful choice of an appropriate interface (usually more than one); aggressive management of secretions and maintenance of effective cough; a continuum of care, training and support to patients, families and caregivers.

C93 CARER INFLUENCE ON NON-INVASIVE VENTILATION TREATMENT IN MOTOR NEURONE DISEASE

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Keywords: caregiving, NIV treatment, resilience

Background: Caregiving plays a significant and central role in the management of motor neurone disease (MND). Patients come to rely on substantial physical assistance with almost every activity of daily living. Although there is a paucity of research describing caregiving in MND, the extant data points to reduced quality of life, increased distress and negative impacts on health for carers. Caregiver variables, as well as patient variables, will predict patient outcomes, including compliance with treatment options. During the course of MND patients may be offered non-invasive ventilation (NIV) to ameliorate breathing difficulties, prolong survival, and improve quality of life. However, not all patients accept this treatment. Knowing that NIV treatment option requires carers that are willing and able to help, an important question is whether the caregiving situation influences uptake of NIV treatment.

Objectives: To measure caregiving variables at the time when the possibility of NIV treatment is presented to someone with MND and his or her carer to identify predictors of those who decline NIV treatment.

Methods: Thirty-five dyads were recruited to the study at the time of assessment for ventilatory support. Seventeen patients went on to accept NIV treatment, and 11 patients declined or were not tolerant of NIV treatment. Seven patients did not yet need NIV, so were excluded. Dispositional and illness-related measures were collected for quantitative analysis using data taken as close to the NIV decision date as possible to minimise differences in the illness situation.

Results: There were no differences in patient illness variables between the NIV group and the no-NIV group. Levels of carer distress were high across both groups; the only significant difference in means was higher anxiety in no-NIV carers ($t = 2.07$, $p < 0.05$). Caregiver health was negatively affected in both groups, particularly emotionally and psychologically with SF36 means significantly below norms; additionally no-NIV carers scored significantly lower for *social functioning* ($t = -2.10$, $p < 0.05$). For dispositional variables: carers in no-NIV dyads were more *neurotic* ($t = 2.06$, $p < 0.05$), and less *resilient* overall ($t = -2.42$, $p < 0.05$), and with respect to *commitment* ($t = -2.51$, $p < 0.05$). A stepwise regression analysis indicated that *resilience: commitment* was the single significant predictor of the use of NIV, explaining over 20% of the variance.

Discussion and conclusions: In line with other research, we found that levels of carer distress are high in MND, and that

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health is negatively affected. Additionally we found a difference in the two NIV groups with respect to dispositional variables: *commitment* - which is described as being involved rather than alienated from aspects of one's life, served to predict uptake of NIV treatment. Some carers may be more naturally inclined to find purpose in their situation; the meaning of the illness situation impacts upon the management of MND.

C94 TRADITIONAL VENTILATION MONITORING MAY UNDERESTIMATE THE VENTILATOR REQUIREMENTS OF MOTOR NEURONE DISEASE PATIENTS

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Keywords: NIV, patient-ventilator interaction, oximetry

Background: Non-invasive ventilation (NIV) is a recommended treatment option for selected patients with motor neurone disease (MND) with ventilatory failure because of its positive impact on survival. However, what constitutes optimal ventilation remains undefined. Most centres assess adequacy of ventilation with overnight pulse oximetry at regular intervals (Leger *et al*. 2002). Modern "domiciliary" ventilators possess technology recording aspects of ventilation such as leak, triggering and Minute Ventilation (MV) (Rabec *et al*. 2009). It is unclear if analysis of Patient-Ventilator interaction (PVI) ultimately yields useful additional information over that gained from measuring pulse oximetry.

Objectives: This prospective study analyses patient-ventilator interaction in longitudinal fashion over a 12 month period in MND patients following initiation of NIV and relationship to nocturnal oximetry and disease state as assessed by ALS-FRS, ALSAQ40, and HADS at 2 time points: 4-6 months post initiation (Point A) and 10-12 months (Point B) post initiation of NIV.

Methods: Ten subjects (mean age 62 (SD 10) years; 9 male) had at least 12 months follow up on NIV and thus were analysed for this study. In all cases, NIV was administered without supplementary oxygen. All were ventilated with a Respironics© Synchrony 2 ventilator; PVI was performed by interrogation of the Encore© Smartcard storage.

Results: In the group, the mean ALS-FRS score fell significantly between points A (27 SD(5.7) and B (23.6 SD (7.68) $t = 2.78$; $p = 0.021$), MV falling in 5 subjects (range -1.14 to -3.53 l/min). A significant correlation was found between MV and ALS-FRS score at points A ($r = 0.74$; $p = 0.014$) and B ($r = 0.65$; $p = 0.043$). At point A, MV correlated significantly with ALS-AQ40 total ($r = -0.63$; $p = 0.05$) and ALS-AQ40 Mobility domain ($r = -0.74$; $p = 0.014$). Tidal volume (TV) fell in 5 subjects (range -3 to -5 l/min) from points A to B. TV correlated significantly with ALS-FRS at point A ($r = 0.70$; $p = 0.025$). In the group, the median oxygen saturation on pulse oximetry was 94% at both time points following initiation of NIV (Point A IQR 89-95%; point B IQR 91-95%). In 7 subjects, pulse oximetry showed < 20 minutes spent below 90% saturation during points A and B suggesting adequate ventilation. Triggering also fell but no association was seen between MV ($r = -0.26$; $p = 0.46$) and Triggering ($r = -0.32$; $p = 0.40$) with adequate oximetry at points A and B. Mask leak did not correlate with MV or ventilator triggering.

Discussion: This data suggests that falling minute ventilation is linked to worsening disability in MND patients receiving domiciliary NIV despite apparently adequate ventilation assessed by overnight oximetry. The insensitivity of oximetry is illustrated in that marked decreases in ventilator triggering and minute ventilation did not necessarily translate into "sub-optimal" oximetry.

Conclusion: Analysing patient-ventilator interaction may be an important adjunct to oximetry for clinicians when optimising ventilation in MND.

Grant from Motor Neurone Disease Association UK.

C95 CURRENT AND PRACTICAL UTILIZATION OF DIAPHRAGM PACING IN ALS/MND: FROM PILOT TRIAL EXPERIENCE TO FDA HUMANITARIAN DEVICE APPROVAL INDICATIONS FOR HELPING RESPIRATION

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Keywords: diaphragm, respiration, gastrostomy

Background: Diaphragm pacing (DP) has been proven to be an acceptable alternative therapy to help with respiration in patients with ALS/MND receiving FDA approval in 2011. This report will review the experience of a single institution throughout the trials and highlight the utilization of DP in current clinical practice.

Objective: Review results of DP from trials and describe the 2011 evaluations of patients for suitability and implementation of DP.

Methods: Two sources of patients: 1) Prospective, nonrandomized, controlled, interventional trials under IRB and/or FDA approval for use of DP at a single institution; 2) Clinical use of DP for ALS following humanitarian use designation (HUD) implantation criteria which involves evidence of chronic hypoventilation (MIP less than 60cm H_20, FVC less than 50% predicted, PCO2 greater than 45mm Hg **or** SaO2 less than 88% for 5 consecutive minutes during sleep) with stimulatable diaphragms as shown by radiographic volitional diaphragm contractions or neurophysiologic evaluation with phrenic nerve conduction studies.

Results: From 2005-2009 during three prospective studies 66 subjects were enrolled with 52 being implanted. The key end result of these trials include: DP can be safely implanted; survival with DP from onset is 4.7 years using Kaplan-Meir survival analysis with many patients still surviving; in a case match comparison patients who used DP and non-invasive ventilation (NIV) survived 16 months longer than those using NIV alone; and patients undergoing combined DP and PEG had a 0% thirty day mortality and 70% one year survival. From 2010-2011 in clinical practice 23 patients were evaluated with implantation occurring in 15 patients following the HUD criteria.Evaluations included pulmonary functions tests (FVC and MIP), arterial blood gases, fluoroscopic sniff test, phrenic nerve conduction studies and overnight pulse oximetry.Reasons for not implanting included: no evidence of hypoventilation (1), non-stimulatable diaphragms (4), excessive

secretions where aspiration would to lead to death or tracheostomy before respiratory failure (2) and one patient declined after discussing end of life issues. Current practice includes discussion of end of life issues and cessation of pacing to allow natural death. 80% (12 out of 15) patients had simultaneous PEG and DP. Post-operative DP use involved 5 initial 30 minute sessions and then increased based on patient presumed benefits. DP is always utilized whenever NIV is used to prevent ensuing atrophy of the diaphragm from NIV suppressing diaphragm function. Ongoing DP adjustments are made according to the patients' need.

Conclusion: DP improves survival and delays tracheostomy ventilation. DP is easily integrated into clinical practice with the primary time of implantation at the time of a PEG. Combining both improves the safety of PEG alone and should decrease total health care cost from separate procedures and hospitalizations.

C96 HIGH FREQUENCY CHEST WALL OSCILLATION (HFCWO) IN AMYOTROPHIC LATERAL SCLEROSIS (ALS) PATIENTS DECREASES RESPIRATORY INFECTIONS REQUIRING ANTIBIOTICS AND/OR HOSPITALIZATION: A PRE-POST OBSERVATIONAL STUDY

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Keywords: high-frequency chest wall oscillation, VEST

Background: Patients with ALS/MND are vulnerable to respiratory infections due to decreased vital capacity, decreased diaphragmatic muscle function, decreased mobility and difficulty managing secretions.Respiratory care in ALS patients with increasing restricted pulmonary function requires attention to clearing pulmonary secretions. HFCWO therapy gently compresses and releases the chest wall up to 25 times per second. This process creates mini-coughs that dislodge mucus from the bronchial walls, increase mobilization, and move it along toward central airways.The action also works to thin thick secretions, making them easier to clear.The HFCWO can be used in a wheelchair, recliner or in bed, and is very easy to place around a person with impaired mobility. Chaisson, *et al.* (1) and Lange, *et al.* (2) demonstrated respiratory function improvement but did not show an effect of HFCWO on infection rate.HFCWO is used with patients with ALS/MND, although there have been no studies to date reporting utilization or the effect of HFCWO on respiratory quality of life.

Objective: To evaluate in an ALS Clinic setting whether there is a decrease in the prevalence of infections requiring antibiotics and/or hospitalization following HFCWO.

Methods: Data was gathered from calls made to patients and caregivers at 5, 15 and 30 days. A validated 7-question respiratory quality of life scale using a 5 point Likert scale (1-5) was completed at each call. The minutes use per day was queried. Surveillance period per patient pre-HFCWO and post-HFCWO was recorded and infections per month of surveillance per patient were calculated for each patient before and after introduction of HFCWO and compared by Wilcoxon rank tests.

Results: ALS patients (36) patients pre-HFCWO had 0.07 ± 0.13 (SD) infections/month which reduced to 0.02 ± 0.06 infections/month (Wilcoxon ranked test $p = 0.0415$).Daily use of HFCWO was 15.9 ± 10.4 minutes for ALS Clinic patients compared with 16.9 ± 12.6 minutes for mean national compliance assessed by Hill-Rom in non-ALS neuromuscular disease patients. The overall respiratory quality of life score was 4.1 (range 3-5) for our ALS patients compared with the national mean 3.8 (range 2-5) for neuromuscular disease patients using HFCWO.

Conclusion: HFCWO significantly reduces the per-patient infection rate in ALS patients. Observational studies employing pre-post-intervention design can provide important clinically meaningful information supporting certain respiratory interventions in ALS patients. HCFWO is a beneficial and effective airway clearance treatment for the patient with ALS/MND.

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C97 MECHANICAL INSUFFLATION-EXSUFFLATION IMPROVES INFLAMMATORY PANEL IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: mechanical insufflation-exsufflation, high sensitive C-reactive protein, serum biomarker

Background: Various biomarkers including serum proteins, cerebrospinal fluid proteins and neuroimaging or neurophysiology studies are currently being identified to make an early diagnosis, monitor the disease progression or measure early therapeutic effects in ALS. Although expectation is high, some of the potential biomarkers are not fully understood in terms of the process behind their abnormal levels. Mechanical Insufflation-Exsufflation (MIE) (CoughAssist: Phillips Respironics) is a noninvasive therapy that removes secretions in patients with an ineffective ability to cough (peak cough flow < 270 L/min). It also expands the lung and reduces atelectasis and promotes airway clearance, so could reduce the frequency of respiratory complications, thereby reducing the rate of respiratory-related hospitalizations in patients with tetraplegia.

Objectives: We aimed to evaluate whether the respiratory condition affects serum inflammatory panels before and after MIE.

Methods: Eight consecutive patients with ALS were enrolled. All patients were hospitalized and in a stable condition, and no procedures were performed within a month. Patients who suffered infections were excluded in this study. Typically patients were waiting for the transfer. We obtain serum samples before and after one week's use of MIE. We measured high sensitive C-reactive protein (hs-CRP), Tumor necrosis

factor alpha (TNF-α), Interleukin-1 beta (IL-1β), Interleukin-6 (IL-6) and pulmonary Surfactant Protein-D (SP-D). We also obtained information on satisfaction with MIE by questionnaires and analyzed if satisfaction was correlated with the improvement of serum inflammation panel. Paired student's t test was used for statistical analysis.

Results: The groups of patients with ALS were associated with higher levels of hs-CRP, IL-6 and SP-D compared to the control groups (hs-CRP 2506 ng/mL: 82 ng/mL, IL-6 2.9 pg/mL: 1.0pg/mL, SP-D 60.3 pg/mL: 17.3pg/mL). MIE therapy for one week significantly reduced hs-CRP (1777 ± 2096 ng/mL (95% CI, 25.1 to 3530, $p = 0.048$)). Patients who do not use non-invasive positive pressure ventilation (NPPV) had higher levels of hs-CRP. MIE decreased the level of hs-CRP more effectively in the patients without NPPV use. No correlations were observed between the patients' satisfaction and hs-CRP reduction.

Discussion: Patients with ALS have higher levels of serum inflammatory panel. It is not fully elucidated why the serum inflammatory markers are elevated. The present study indicated that short-term use of MIE significantly decreased serum hs-CRP levels. This suggests that respiratory complications such as microatelectasis could contribute to an increase in inflammatory proteins. MIE assists removing secretions and expanding alveoli effectively. This could explain why the MIE did not decrease hs-CRP effectively in the patients who had already been using NPPV. Early use of non-invasive ventilation is reported to prolong survival in patients with ALS although the mechanism remains unclear. It could be the key to improve ALS treatments to study further the relationship between the respiratory condition and inflammatory status.

C98 THE EFFECTS OF HIGH FAT VERSUS HIGH CARBOHYDRATE ENTERAL NUTRITION IN ALS PATIENTS WITH RESPIRATORY DYSFUNCTION

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Keywords: respiratory, nutrition, PEG tube

Background: There is evidence to support an increased mortality rate in ALS patients when PEG placement occurs in the setting of ventilatory failure. We propose that the increased mortality may be due in part to the increased metabolic demand on the ventilatory system in the post-prandial state.

Objectives: 1) To examine the respiratory effects of high carbohydrate enteral feeding formula (Nutren); 2) To determine if A) slow (continuous) enteral feeding or B) high fat enteral feedings (50%, Pulmocare) would avoid the increased respiratory demands on ALS patients with ventilatory dysfunction.

Methods: We prospectively examined minute ventilation, respiratory rate, tidal volume, end-tidal CO_2, CO_2 elimination rates and arterial blood gases in the fasting state and one hour after a high fat or high carbohydrate bolus or high carbohydrate continuous enteral feeding in ALS patients with PEG placement and ventilatory dysfunction.

Subjects: Eleven subjects age 18-80 with definite or probable ALS who met the following criteria: 1) PEG placed for the treatment of dysphagia/undernutrition; 2) FVC $< 70\%$ predicted or $PaCO_2 > 45$ mmHg or nocturnal oxygen desaturation to 88% for > 5 minutes were enrolled. The primary outcome measure was the mean difference between $PaCO_2$ in the fasting and post-prandial states.

Results: Statistical analysis used a one-sided signed rank test. All statistical results were obtained using SAS Version 9.1.3. Demographic measures included mean age (68.8 years), mean BMI (26.0), and mean weight (72.8 kg) for the10 patients given high carbohydrate bolus feeding. Mean days between PEG placement and day of visit (22.6), and between PEG placement and feeding (2.9 days) were calculated for all 10 patients receiving bolus high carbohydrate enteral feeding. Differences in fasting and post-prandial state were obtained for the variables: $PaCO_2$, minute ventilation, respiratory rate, tidal volume, end-title CO_2, CO_2 elimination, FVC %, FVC (L), PaO_2, pH, NIF %. Because of the small sample size of ten patients, all differences were tested for significance using a t-test. Respiratory rate ($p = 0.021$), CO_2 elimination ($p = 0.01$), and pH ($p = 0.01$) were found to be significantly increased in post-prandial state compared to the fasting state in the bolus high carbohydrate group ($N = 10$).

CO_2 elimination rate did increase during continuous feeding of high carbohydrate formula, but not after a high fat bolus. There was no difference in respiratory rate during continuous feeding of high carbohydrate or after high fat bolus feeding in the patient ($N = 1$) studied thus far.

Conclusions: 1) Respiratory rate, minute ventilation, and CO_2 elimination increased after enteral bolus feedings, adding to the work of breathing. 2) Continuous slow enteral feeding or high fat, low carbohydrate feedings may place less respiratory demands on the ALS patient with ventilatory failure. 3) Further study in a large cohort of patients is needed to determine the clinical significance of these results.

SESSION 12 JOINT CLOSING SESSION

C99 ALS RESEARCH AND TREATMENT: WHERE TO FROM HERE?

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The last decade has seen unprecedented progress in ALS research and care. However, disease modifying therapy of major effect is still an aspiration rather than a reality. We now understand that ALS is not a single disease with one cause, but a clinicopathological syndrome resulting from a complex convergence of genetic susceptibility, age-related damage and possible environmental influences. The precise biological pathways underlying this complexity are beginning to emerge from genetic studies and neuropathology.

The combination of larger genome-wide association studies, whole exome sequencing and RNA expression studies will rapidly expand the number of genes which are implicated in ALS, for both familial and sporadic cases. Almost two decades after the identification of the first genetic cause of ALS (SOD1), we still do not know the exact disease mechanism. Therefore the major challenge is how to extract meaningful biological insights into ALS from the resulting huge quantity of data. To exploit this rapid expansion in information for the benefit of patients, sophisticated pathway analysis is required to produce points of convergence which can serve as targets for therapy. Better pre-clinical models of disease, including stem cell derived human motor neurons will be widely available to test the relevance of these pathways. However, a complete description of the origin of ALS and the nature disease heterogeneity requires an understanding of the disease as a system failure, not just as a disorder of a specific population of cells.

Most patients entering clinical trials have well established disease. It is imperative that we find ways of identifying and studying the disease in its earliest, potentially most therapeutically tractable, stages. To this end international efforts to coordinate biomarker studies and detailed epidemiology to understand whether specific populations are at risk are also critical. In combination with multidisciplinary, proactive management of nutrition, respiratory function and disability, multimodal treatment of ALS in its earliest phases offers a tangible hope for making an impact in what is one of the most challenging diseases known to medicine.

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THEME 1 RESPIRATORY AND NUTRITIONAL MANAGEMENT

P1 LONG TERM INVASIVE VENTILATION THROUGH TRACHEOSTOMY IN ADVANCED ALS: CAROLINAS NEUROMUSCULAR/ALS-MDA CENTER EXPERIENCE

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Keywords: invasive ventilation, tracheostomy, survival

Background: Amyotrophic lateral sclerosis (ALS) is a life-limiting disease with 50% patients dying within 3 years and 90% within 5 years after diagnosis. Although invasive ventilation through tracheostomy (IVTT) may significantly extend the survival in ALS patients with respiratory insufficiency, it is not commonly recommended due to perceived poor quality of life, high cost of care, and clinicians' personal biases.

Objectives: Identify life expectancy, quality of life, and specific challenges of ALS IVTT patient management imposed on caregivers, physicians, and multidisciplinary clinic staff.

Methods: We audited medical records of IVTT patients followed at Carolinas Neuromuscular/ALS-MDA in the Department of Neurology at the Carolinas Medical Center during the past 3 years. We reviewed several patients' factors: gender, race, age at onset, clinical manifestation site, time between symptom onset and tracheostomy, time after tracheostomy, and total ALS FRS-R score and respiratory ALS FRS-R subscore.

Results: 42 IVTT patients evaluated at CMC ALS Clinic since beginning of 2007 were identified: 26/42(60%) males, 16/42(40%) females; 7/42(17.5%) African Americans, 2/42(5%) Hispanics, and 33/40(77.5%) Caucasians. 12/42(29%) patients had bulbar, 16/42(38%) upper extremity, 11/42(26%) lower extremity, 2/42(5%) respiratory, and 1/42(2%) PLS onset. The age of symptom onset was 26-68(mean 53.6) years; the age at tracheostomy procedure ranged 26-72(mean 56.6) years. The time between symptom onset and tracheostomy procedure was 0.3-15 (mean 3.4) years. Time after tracheostomy in 19 living patients was 0.7-13(mean 3.9) years; time between tracheostomy and death in 22 deceased patients was 0.4-6.4(mean 2.44) years. 40/42(95%) patients had PEG tubes. 24/42(57%) had ALS FRS-R 0-3; 10/42(24%) had ALS FRS-R 4-10; 1/42(2%) had ALS FRS-R of 36; no ALS FRS-R was available for 7/42(17%). 4/42(10%) patients developed true locked in state. Only one IVTT patient requested hospice assisted withdrawal of life support.

Discussion: IVTT prolongs survival in ALS patients with respiratory failure but it brings a great financial and care burden. There have been only a few studies that have investigated the outcome of ALS patients receiving treatment with IVTT, and there is little knowledge about the clinical characteristics of the ALS patients with long term IVTT. 42/300(14%) of all ALS patients followed at CMC ALS Center during 2007-2011 were on IVTT. More than half patients had ALS FRS-R 0-3. Our experience stresses the importance of careful planning of medical interventions, and providing counseling for patients and families in making decisions about initiation as well as withdrawal of ventilatory support.

Conclusions: IVTT is an effective measure to prolong survival in ALS. More studies are needed to evaluate the quality of life in long term IVTT ALS patients. Timely initiation of discussions about end of life care to facilitate decision-making process with patients and their caregivers is an important part of multidisciplinary care.

P2 THREE-MONTH SURVIVAL AFTER AN ICU ADMISSION FOR ACUTE RESPIRATORY FAILURE IN AMYOTROPHIC LATERAL SCLEROSIS (ALS) AND PROGNOSTIC FACTORS

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Keywords: respiratory insufficiency, intensive care, acute

Background: The main complication of ALS is chronic respiratory failure due to muscle weakness. Non-invasive ventilation (NIV) improves survival whilst maintaining quality of life. However, during an episode of acute respiratory distress, the decision of whether or not to admit the patient to the ICU can be a difficult one to make.

Objective: To describe the survival of patients with ALS 3 months after an ICU stay and identify prognostic risk factors.

Methods: A descriptive, observational, retrospective monocentric study in the ICU of the Department of Respiratory and Intensive Care Medicine of a 1600-bed teaching hospital,

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participating to the ALS reference center in Paris, France. All the first ICU stays of ALS patients were analyzed, except when they were pre-programmed (e.g. for tracheostomy or at risk gastrostomy). The variables analysed fell within three categories: 1) disease description and neurologic status within the 3 months preceding the admission; 2) presence or not of tracheotomy or of non-invasive ventilatory support (NIV) before the ICU stay; 3) ICU stay description. The analysis was first conducted in the whole population, and then restricted to the NIV group.

Results: Between 1st January 2000 and 30th June 2009, 111 admissions of ALS patients were recorded. Of these, 90 corresponded to the inclusion criteria, in 66 men and 24 women. The overall 3 months survival was 50%. It was not associated with any neurological descriptor, including the existence of a bulbar involvement. A prior history of domiciliary ventilation did not affect 3-month mortality either. Age and the severity of respiratory acidosis at admission were independent predictors of the 3-months mortality according to the multivariate analysis of the data. A tracheotomy either before or during the ICU stay was associated with better survival. These results were identical in the "prior NIV" subgroup. One third of the patients were alive after one year.

Conclusion: In this retrospective cohort, age and respiratory acidosis were the only factors that predicted the risk of death at 3 months. The lack of predictive value of the bulbar involvement came as a surprise but could be the result of a strong referral bias. The median survival following the admission of an ALS patient to the ICU is short (median of 4 months), but one third of the patients are still alive at one year. This indicates that systematically censoring ICU admission in ALS patients, even if they suffer from established respiratory insufficiency, is unwarranted.

P3 ANALYSIS OF THE END OF LIFE SITUATION IN 115 CONSECUTIVE ALS PATIENTS IN THE PAST NINE YEARS

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Keywords: NPPV, end of life, morphine

Background: Recently ALS treatment has been changing in Japan due to the wide use of NPPV and the use of morphine.

Objectives: To clarify the change of the end of life situation by the spread of NPPV and the use of morphine.

Methods: In 115 cases who died by ALS from April, 2002, we examined the use of NPPV, use of morphine and situation of the end of life.

We compared two periods : from April 2002 to March 2005 (1st period); and from April 2005 to March 2011 (2nd period).

Results: Among eight patients using permanent mechanical ventilation, six of them died of infection and 2 of them died by accident. In the remaining 107 cases, there were 30 cases in the 1st period and 77 cases in the 2nd period.

NPPV was used in 5 cases (17%) in the 1st period and 44 cases (57%) in the 2nd period, morphine was used in 1 case (3%) and 37 cases (48%), and sudden death occurred in 9 cases (30%) and 10 cases (13%), respectively. No case of sudden death used NPPV and morphine. Almost all of the patients who used NPPV needed to use morphine. Almost all of the patients who used morphine died peacefully, except for one patient who needed terminal sedation.

Discussion: It was reported that approximately 50% of ALS patients died suddenly before NPPV use spread. In this study sudden death was obviously much higher in 1st period when NPPV was not popular as compared to 2nd period. We assume that the use of NPPV avoids sudden death even in the patients who had bulbar symptoms. NPPV prolongs one's life, then patients come to feel dyspnea for a longer period than without NPPV. We should not hesitate to use morphine especially for cases with NPPV. Further investigation is needed to clarify whether NPPV is useful to prevent sudden death even for the patients who have severe bulbar symptoms and mild respiratory dysfunction which does not satisfy the criteria for indication of NPPV.

Conclusion: NPPV improves not only QOL and the life expectancy but also prevents sudden death, therefore the patient can spend the end period with their family. Since neurologists see ALS patient until the end stage in Japan, to make that period meaningful, we need to become proficient in using NPPV and morphine.

P4 AN EXPLORATORY MIXED METHODS STUDY OF PATIENTS WITH MOTOR NEURONE DISEASE WHO ARE ON NON-INVASIVE VENTILATION

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Keywords: NIV, longitudinal

Background: Non-invasive ventilation (NIV) has been recommended as a treatment option for some patients with motor neurone disease (MND) because of its positive impact on survival. However, previous studies do not provide insight into how patients' perceptions of NIV may evolve over time, and how this influences NIV use.

Objectives: To investigate longitudinally how NIV is experienced by MND patients while their condition deteriorates.

Methods: Five MND patients (male = 4, mean age = 59 years) were serially interviewed every three months. Interviews were transcribed verbatim and analysed using interpretative phenomenological analysis (IPA). These patients were part of a wider study, the data presented focuses on all participants for whom we have at least four post NIV interviews, covering more than 12 months of NIV treatment (mean = 13 months), along with ventilator interaction data.

Results: The IPA identified three main themes: physical-psychological relationship, coping style effects and the impact of the terminal prognosis. The first theme explains how physical benefits of NIV were related to psychological benefits, while no relationship between negative physical aspects of NIV and psychological effects was observed. The second theme focuses on participants' coping styles and their experience of NIV; positive experience of NIV was reported among

patients with active coping styles, who also expressed their positive attitude towards life, while an avoidant and resistant attitude towards NIV was found with a patient who displayed the same attitude towards the illness. The final concept describes how the sense of death or the sense of hopelessness both provoked by the perception of MND, prompted a reinforced or a reluctant engagement with NIV, respectively. The ventilator interaction data identified regular use of NIV by four participants (mean NIV use time = 9 hrs 20 mins), who had positive coping styles, and irregular use by one participant (mean NIV use time = 2 hrs 30 mins), who had a negative coping style and in whom MND induced feelings of hopelessness.

Discussion: Generally, patients displayed psychological benefits from NIV and these appeared to increase over time. However, individuals may be resistant to NIV because they perceive a hopeless future and due to their coping styles. The study suggests that the individual's experience of NIV is determined by their interpretation of the illness and its perceived impact on the future, which is in turn influenced by their coping styles and their attitude towards life. The qualitative interpretations were in agreement with the pattern of participants' NIV use.

Grant from Motor Neurone Disease Association UK.

P5 HOW DOES THE "REAL-LIFE" PRACTICE OF NON-INVASIVE VENTILATION IN AMYOTROPHIC LATERAL SCLEROSIS AT A LARGE REFERENCE CENTER COMPARE WITH CONSENSUS EXPERT RECOMMENDATIONS?

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Keywords: registry, intermittent positive-pressure ventilation, respiratory insufficiency

Background: Chronic respiratory failure due to respiratory muscle weakness is frequent in amyotrophic lateral sclerosis (ALS) and a leading cause of death. Non-invasive ventilation (NIV) is a recognized standard of care that alleviates symptoms and prolongs survival. Criteria for starting NIV in ALS derive from expert recommendations, but the evidence behind them is weak, and how realistic they are in practice is unknown.

Objective: To describe the practice of the main French tertiary referral centre for ALS with regard to NIV initiation in reference with the corresponding French expert consensus guidelines.

Methods: The French national register of ALS tertiary referral centres (17 centres in France) contains data on 5410 patients followed up in the Paris centre since 2004. Among these, 594 patients have been started on NIV. We extracted the main criterion identified by the clinicians as the reason to initiate NIV from the database (among symptoms, $PaCO_2$, VC, Pimax, SNIP and the percentage of night time spent with a $SpO_2 < 90\%$) and described the patients accordingly.

Results: Symptoms were the main reason for NIV initiation (39%; only reason in 6 cases), followed by hypercapnea (28%). Functional respiratory impairment rarely came first (Pimax or SNIP in 3%; VC in 2%; nocturnal desaturation 3%). 10% were ventilated due to acute respiratory insufficiency. Sixty-five patients (11%) were ventilated without demonstrating any of the consensus criteria for starting NIV. The main reason for 4% of the patients was a high blood level of HCO_{3-}.

At the time of NIV initiation, ninety percent of the patients reported symptoms (effort dyspnoea, dyspnoea at rest, orthopnoea, nocturnal arousals, daytime somnolence or morning headaches). Paradoxical respiration was observed in 47% of patients and use of accessory muscles in 70%. Average VC was 52 + 22%, average Pimax was 56 + 33%, average SNIP was 41 + 28, average nocturnal desaturation time was 32 + 33 % of the time spent <90%.

Conclusion: At the time of starting NIV, our patients were very symptomatic and often hypercapnic, and had functional characteristics suggesting that NIV would have been started earlier if guidelines had been applied rigorously. The main hypothesis to explain these observations is an insufficient resource allocation to the respiratory management of ALS, in spite of the fact that our center has 3 full time respiratory physicians devoted to this activity and the highest national proportion of ALS patients receiving NIV.

In collaboration with the ALS/NMD group of Société de Pneumologie en Langue Française

P6 EARLY TREATMENT WITH NONINVASIVE POSITIVE PRESSURE VENTILATION PROLONGS SURVIVAL IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Keywords: noninvasive positive pressure ventilation, pulmonary ventilation function, forced vital capacity

Objective: To investigate 1 year survival of ALS patients with FVC < 75% treated with NPPV, compared to a well-matched population of ALS patients, who refused or were intolerant to NPPV.

Methods: We investigated 60 consecutive ALS patients who underwent pulmonary function tests. Twenty-eight patients presented a FVC > 75% and served as control group, 33 patients presented a FVC < 75% and showed nocturnal respiratory insufficiency, requiring NPPV; 14 patients who were treated with NPPV constituted group 1, while 12 patients who refused or were intolerant constituted group 2.

Results: Increased survival rate at 1 year in patients with FVC < 75% treated with NPPV, as compared to those who refused or could not tolerate NPPV ($P < 0.05$), but showed no difference with the control($P > 0.05$). The median rate of decline in FVC% was slower in NPPV patients than in patients who did not use NPPV.

Conclusions: Early treatment with NPPV delays the development of the patient's condition and prolongs survival and reduces decline of FVC% in ALS.

P7 HOME BASED UNATTENDED SLEEP STUDY AS AN INTEGRAL COMPONENT OF MULTIDISCIPLINARY ALS CLINIC

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Keywords: sleep disordered breathing, respiratory insufficiency, NIV

Background: The most frequent terminal event in Amyotrophic Lateral Sclerosis (ALS) is respiratory insufficiency (RI) (1). RI becomes evident earlier during sleep rather than daytime (2) with resultant fatigue, excessive daytime sleepiness and poor quality of life (3). Early diagnosis of RI is important because noninvasive ventilation is a standard of care in ALS with increased survival related to number of ventilator use hours (1).

Objectives: To capture SDB (sleep disordered breathing) causing RI and identify patients needing NIV at their earliest possible physician-patient interface by a home based unattended sleep study, as an integral component of a multidisciplinary ALS clinic.

Methods: Peripheral arterial tonometry (PAT) is a noninvasive technique to measure changes in blood flow to the finger, an area nearly exclusively regulated by adrenergic innervation. PAT signal changes in response to airway obstruction and arousals. Algorithms of periodicity, duration, heart rate and desaturation (4,5) indirectly estimate AHI (Apnea Hypopnea Index) by identifying surges of sympathetic activation (autonomic arousals) occurring at termination of respiratory events. Advantages include home based setting, simplicity of equipment, reduced cost and it incorporates Actigraphy calculating total sleep time providing 'events per hour in sleep' rather 'events per hour in bed' (6). We used Watch PAT100 (Itamar Medical) which had showed good correlation between AHI measured with simultaneous PAT100 and standard laboratory PSG setting (rho = 0.88).

Results: 15 patients were studied over 3 months. Sitting and supine FVC (Forced Vital Capacity), NIF (Negative Inspiratory Force), sleep efficiency, AHI (Apnea Hypopnea Index), RDI (Respiratory Disturbance Index) and ODI (Oxygen Desaturation Index) were reviewed. 9 were males (60%), 6 females (40%). 3/15 (20%) showed sitting FVC $< 50\%$ qualifying for NIV. 12/15 (80%) showed sitting FVC $> 50\%$. One showed supine FVC $< 50\%$. Out of 11/15 (73.3%) who could not qualify for NIV based on FVC $> 50\%$ (sitting or supine) or NIF (< -60); 7/11 (63.6%) showed elevated AHI (> 5), 2/11 (18.1%) showed elevated RDI, 9/11 (81.8%) showed elevated AHI and/or RDI (> 5) and qualified for NIV. Out of 11/15 (73.3%) who could not qualify for NIV based on FVC $> 50\%$ (sitting and supine); 8/11 (72.7%) showed nocturnal O2 desaturation nadir $< 90\%$, while all these 8 patients showed diurnal resting O2 $> 90\%$.

Discussion and conclusions: ALS patients have difficulty tolerating multichannel sleep laboratory studies because of weakness, reduced mobility, secretions, dysarthria hampering communication with technologist in addition to "first night effect". An alternative approach is home based unattended sleep study by peripheral arterial tonometry; an easy and cost effective method (8) to identify patients early for NIV as standard diurnal respiratory measures like FVC and NIF can undermine degree of RI. We propose that unattended home based sleep studies should be an integral part of a multidisciplinary ALS clinic.

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P8 THE SURVIVAL OF AMYOTROPHIC LATERAL SCLEROSIS PATIENTS PLACED UNDER NON INVASIVE VENTILATION (NIV) DOES NOT DEPEND ON THE VENTILATORY MODE USED (VOLUME VS. PRESSURE NIV)

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Keywords: intermittent positive ventilation, volumetric ventilation, barometric ventilation

Background: Non-invasive ventilation (NIV) has become a universally accepted standard of care in amyotrophic lateral sclerosis (ALS) when the disease-related respiratory muscle weakness is responsible for chronic respiratory failure. NIV alleviates symptoms and prolongs survival. Whether or not these benefits depend on the ventilatory mode used to deliver NIV is not precisely known.

Objective: To compare survival in two separate cohorts of ALS patients treated at two distinct European tertiary referral centres, with pressure preset NIV in one case and volume preset NIV in the other case (p-NIV and v-NIV, respectively).

Methods: Retrospective comparison of 62 patients receiving v-NIV (Valencia, Spain) with 82 patients receiving p-NIV (Paris, France), in terms of their anthropometric characteristics, neurological data, respiratory variables, and 5 years survival. Of note, the two centers follow the same clinical management guidelines, the only important difference being their choice of ventilatory mode.

Results:: There was no statistically significant difference between the two groups at the time of NIV initiation, regarding age (62 ± 9 vs 64 ± 11 years in the p-NIV vs v-NIV group, respectively), vital capacity (52 ± 24 vs 55 ± 19 % pred), Pimax (47 ± 25 vs 42 ± 25 cmH20), $PaCO_2$ (51 ± 9 vs 53 ± 9 mmHg) and total night time spent with a $SpO_2 < 90\%$ (TST90%, 35 ± 30 vs 38 ± 33 %). The Norris bulbar score was lower in the p-NIV group (27 ± 10 vs 24 ± 5, $p < 0.05$). The median survival from NIV onset was 15 ± 4 months in the v-NIV cohort, vs. 17 ± 4 months in the p-NIV cohort ($p = 0.4$). Restricting the analysis to patients with a bulbar onset of the disease ($n = 23$ in the v-NIV cohort, $n = 9$ in the p-NIV cohort, $p < 0.05$), who are often said to be more difficult to ventilate, provided similar results (8 ± 2 months in the v-NIV cohort, vs. 9 ± 1 months in the p-NIV cohort, $p = 0.27$). A non-significant trend toward a better survival was observed in the bulbar onset patients receiving v-NIV after the first year of NIV. A multivariate analysis conducted on the population as a whole ($n = 144$) identified usual prognostic factors for survival, namely an older age (HR = 1.03, $p = 0.01$), vital capacity (HR = 0.5, $p = 0.01$) and a bulbar onset of the disease (HR = 0.4, p = 0. 008). In addition, this analysis also identified $PaCO_2$ under NIV as an independent prognostic factor (HR = 1.1, $p = 0.009$), irrespective of the ventilatory mode used.

Conclusion: p-NIV and v-NIV provide similar survival in ALS patients with chronic respiratory insufficiency. Failure to lower $PaCO_2$ with NIV is an independent prognostic factor. This suggests that an adequate NIV is more important than the ventilatory mode used to deliver it.

P9 IMPACT OF THE QUALITY OF NON-INVASIVE VENTILATION ON THE ONE-YEAR MORTALITY OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: intermittent positive pressure, home ventilation, nocturnal monitoring

Background: Non-invasive ventilation (NIV) is the standard of care of ALS-related chronic respiratory insufficiency due to respiratory muscle weakness. It improves quality of life and improves survival. There are data to suggest that this benefit is lost in when NIV is ill-tolerated by patients.

Objectives: To evaluate the impact of the quality of NIV on the one-year survival of ALS patients.

Methods: We analysed survival rates during the first year following the introduction of NIV in 82 patients with ALS. The quality of NIV was evaluated at one month. Patients who had a $SpO_2 < 90\%$ for more than 5% of the time were considered inadequately ventilated and constituted Group 1. They were compared with a Group 2 made of the patients not exhibiting nocturnal desaturations, in terms of 1) symptoms; 2) sleep nocturnal polygraphic recordings (Reslink®, Resmed, Sydney, Australia); 3) arterial blood gases.

Results: Group 1 comprised 42 patients (51%). Group 1 and Group 2 were generally similar, without significant differences in terms of variables known to be associated with poor prognosis (older age, bulbar onset, vital capacity and its decline rhythm, rapid functional decline, ALSFRS). Inadequate ventilation was due to air leaks (56%), upper airway obstruction (24%) and other causes (20%). The one-year survival was significantly better in Group 2 than in Group 1 (12 vs 10.5 months, $p = 0.002$). In a multivariate analysis, an inadequate NIV at one month appeared as an independent predictor of mortality (HR = 2.32, $p = 0.029$), in addition to the body mass index (HR = 0.09, $p = 0.001$), vital capacity (HR = 0.97, $p = 0.010$), bulbar onset (HR = 4.31, $p = 0.002$) and a rapid disease progression (HR = 3.55, $p = 0.014$).

Conclusions: Poor quality of ventilation at one month is an independent risk factor for mortality at one year in ALS patients.

P10 DEPRESSED VENTILATORY DRIVE WITH GLOTTIS CLOSURE: A NEWLY RECOGNISED CAUSE OF NIV INTOLERANCE IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: obstructive apnea, intermittent positive-pressure ventilation, respiratory insufficiency

Background: Non invasive ventilation (NIV) can be rendered ineffective by various mechanisms: 1) air leaks; 2) airway obstruction; 3) reduced ventilatory drive; 4) patient-ventilator asynchrony. Depressed ventilatory drive with glottis closure can occur during sleep in response to hyperventilation. It has been described in normal subjects, but never in ALS patients and never as a cause of NIV inefficiency.

Objectives: To describe and elucidate the occurrence of depressed ventilatory drive with glottis closure in ALS patients unable to tolerate NIV and presenting with signs of airway obstruction.

Methods: All the ALS patients treated with NIV in the Paris ALS reference centre (France) are followed up with nocturnal monitoring every 3 months (automatic polygraphy, Reslink™ Resmed, Sydney Australia). A full in-lab sleep polygraphy is performed under NIV when episodes of airway obstruction are retained as the main cause of a NIV inefficiency. We therefore reviewed the 2010 database of our sleep center to identify episodes of depressed ventilatory drive with glottis closure. They were defined as an event (desaturation

or/ad arousal) due to an interrupted or reduced inspiratory flow associated with a decrease in ventilatory drive (Gonzalez-Bermejo, Thorax 2011).

Results: Of 1528 nocturnal monitorings performed in 2010, 213 were carried out on ALS patients. Fifteen of these demonstrated depressed ventilatory drive with glottis closure. The absence of hypocapnea ruled out a hyperventilation secondary to the NIV. The impact of this phenomenon was significant with nocturnal desaturations in 9 patients (12 % (1-18) of the recording time) and arousals in 6 patients. We observed that the phenomenon occurred during waking hours in two patients, thereby allowing bronchoscopic visualisation. The lower jaw was seen to fall, the tongue slid backwards and the epiglottis fell onto the glottis which, in all, created a total closure of the upper airway. This obstruction was accompanied by a complete absence of respiratory movements. Changing NIV settings failed to solve the problem. Mandibular advancement with NIV provided short-term improvements.

Conclusion: Depressed ventilatory drive with glottis closure should be suspected in ALS patients when airway obstruction occurs under NIV. How mandibular advancement can correct this problem is currently being investigated.

P11 LONG-TERM EVOLUTION OF AMYOTROPHIC LATERAL SCLEROSIS PATIENTS UNDERGOING CHRONIC DIAPHRAGM CONDITIONING: A 2 YEAR FOLLOW-UP OF THE FRENCH ALS-PHRENIC PACING COHORT

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Keywords: intermittent positive ventilation, phrenic nerve pacing, diaphragm

Background: The effects of diaphragm conditioning through phrenic nerve stimulation with a laparoscopically implanted intramuscular stimulator (NeurRxDP4, Synapse Biomedical, Oberlin, OH, USA) on the decline of vital capacity (VC) in ALS have been tested during a prospective, non-controlled, non-randomized, multicenter pivotal trial. Preliminary information suggest that diaphragm conditioning can slow down VC decline in certain cases and can improve sleep. Final results are pending. This study involved a 9 months follow-up, beyond which the patients and their caregivers were free to continue using phrenic stimulation. Among the patients enrolled at the French center of this study (n = 18, the last one has been implanted in March 2009), 17 patients continued to pace their diaphragm after completion of the study follow-up (for more than 6h/day in 16 cases, less than 1 hour in one) and one stopped.

Objective: To assess the status of ALS patiens still using their phrenic stimulator 2 years after the completion of the initial study.

Methods: Comparison of the survival observed in the implanted patients (n = 18, and 11 with an onset of disease after 2006) with survival data gathered prospectively at our center since 2006 (onset of disease after 2006, n = 756). The effect of stimulation on the survival of patients was assessed using a Cox regression model with stimulation as time-dependent variable.

Results: At the time of implantation, the implanted patients were 66 + 7.71, 55 % were men, 9% had had a bulbar onset. Their ALSRS-r total score was 31 + 6, with riluzole use in 100% of cases, and non-invasive ventilation use in 50%. As of April 2011, 3 patients (all implanted during the last trimester of 2008) were still alive, 2 with NIV at a stable dose of 8 hours/night, 1 without NIV. The hazard ratio for death at the 2 years time point was 5.46 for the non-implanted patients vs. the implanted ones (95% CI 0.732; 40.788, p = 0.09).

Conclusion: Diaphragm conditioning with phrenic stimulation might prolong survival and prevent the progression of ventilator dependency in ALS. These outcomes must be studied in a randomized controlled manner.

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P12 CLINICAL IMPLICATION OF PARAMETERS USED FOR EVALUATING EXPIRATORY MUSCLE FUNCTION IN NMD

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Keywords: expiratory muscle, peak cough flow, peak expiratory flow

Background: Pulmonary complication caused by respiratory muscle weakness comprises major mortality in neuromuscular disease (NMD). Expiratory muscle weakness not only results in a reduction of coughing capacity but also induces a failure of clearing airway secretions. Therefore, assessment of expiratory muscle function is important to infer coughing capacity of NMD patients.

Objectives: The aim of this study is to evaluate clinical utility of several parameters used for assessing expiratory muscle function; peak expiratory flow (PF), peak cough flow (PCF), and maximal expiratory pressure (MEP).

Methods: Thirty amyotrophic lateral sclerosis (ALS) patients with bulbar impairment and 31 Duchenne muscular dystrophy (DMD) patients were included in this study. 53 age-matched healthy subjects were included as a control group. Control group A were age-matched with DMD, and control group B were age-matched with ALS patients. PF and PCF were obtained with a peak flow meter. MEP was measured by a respiratory pressure meter with mouth piece.

Results: Mean MEP in DMD and ALS was 23.8 ± 14.6% and 18.2 ± 15.1% of normal predicted values respectively. Mean PF and PCF was 147.4 ± 46.1 L/min and 205.6 ± 54.9 L/min in DMD, 125.5 ± 46.0 L/min and 153.3 ± 59.1 L/min

in ALS, 490.3 ± 55.2 L/min and 1674.1 ± 59.0 L/min in control group A, and 435.6 ± 52.5 L/min and 636.7 ± 62.8 L/min in control group B respectively. Patient groups showed significantly lower values of PF and PCF than control group ($p < 0.05$). PF and PCF were positively correlated with MEP both in DMD ($r^2 = 0.888$, $r^2 = 0.458$, $p < 0.01$) and ALS patients ($r^2 = 0.997$, $r^2 = 0.456$, $p < 0.01$). The mean PCF-PF difference calculated by a formula of PCF-PF/PF × 100 was 46.2 ± 27.1% in control group A, 47.5 ± 16.3% in control group B, 41.9 ± 15.2% in DMD, and 21.5 ± 7.1% in ALS with bulbar impairment respectively. The PCF-PF difference in ALS with bulbar impairment was significantly lower compared to values of control group ($p < 0.05$).

Discussion and conclusions: Both of PCF and PF were significantly decreased in NMD patient group, and both parameters positively correlated with MEP. The results of this study implicate that these parameters can be useful for assessing expiratory muscle weakness, especially for patients presenting difficulty in measuring MEP. In addition, PCF-PF difference can be applied as a valuable parameter to detect bulbar impairment.

P13 THE EFFECT OF POSTURE ON MAXIMAL PEAK COUGH FLOW VALUES IN INDIVIDUALS WITH ALS

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Keywords: coughing, swallowing, positioning

Background: Peak cough flow (PCF) is a surrogate measure of cough strength and capacity and is measured using a spirometer or hand-held PCF meter. According to American Thoracic Society (ATS) standards, patients should be seated fully upright during pulmonary function testing. However, the effect of sitting angle on PCF in patients with ALS has not been studied systematically.

Objective: The following research questions were of interest: 1) Is there a difference in PCF during coughing when patients with ALS sit with 90 degrees flexion as compared to 80 degrees flexion? 2) What degree of forward flexion do patients with ALS naturally assume while swallowing?

Methods: Ten patients with ALS participated in the pilot study. They ranged in age from 39-75 years with an average time since diagnosis of 18.8 months and a mean total ALS-FRS-R score of 27.9. To answer the first research question, we used a within-group, pre-test post-test design. Measures of PCF were collected with a Vernier digital spirometer and respiratory chest strap while the patient was seated in a modified chair which included a fixed Goneometer attachment and a moveable back support to place patients in each of the two angles of flexion, (90 and then 80 degrees). A digital laser was used to validate the point at which the particular degree of forward flexion was reached. The dependent measure was the maximum of three coughs. For the second research question, patients were observed eating pudding over 10 swallows, while seating in the modified chair. The dependent measure was the average degree of forward flexion at the onset of swallowing.

Results: Research Question 1: The mean PCF at 90 degrees (239.4, SD = 166.1) was less than the mean PCF at 80 degrees (267.6; SD = 151.1); however, this difference was not statistically significant (Wilcoxon Signed Ranks, $z = -1.68$; $p = 0.08$). Seven out of 10 participants showed a pattern of greater PCF when seated at 80 versus 90 degrees. Research Question 2: The mean degree of flexion during eating and swallowing was 85.1 degrees. Eight of the 10 participants had a forward flexion of less than 90 degrees; 2/10 had a forward flexion greater than 90 degrees.

Conclusions: The pilot nature of this study precludes firm conclusions about the effects of forward flexion angle on PCF during coughing. However, the preliminary findings are the foundation for future research with larger samples. The finding that 8/10 of the participants sat slightly forward while swallowing, suggests a natural tendency to compensate for weakness and improve airway protection through postural adjustment. Theoretical and clinical implications will be discussed.

P14 THE VOICE ANALYSIS AND MANOMETRIC EVALUATION OF SWALLOWING IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: manometry, voice analysis, movement disorders

Background: Patients with bulbar onset present firstly with speech disorders, swallowing problems and dysphonia. The type and dynamic of voice disturbances can be assessed via perceptual, videostroboscopy and acoustic analyses. The swallowing problems in ALS patients are frequently estimated using manometric parameters.

Objective: The aim of the study was to analyse the phonation function of the larynx and the disturbances of the oro-pharyngeal swallowing phase of dysphagia in ALS patients.

Material and methods: Thirty two ALS patients were diagnosed in the Department of Neurology. There were 19 (59.4%) male and 13 (40.6%) female patients, aged between 26 and 78 (mean: 59.9 ± 11.4 years). Complete phoniatric and manometric examinations were performed three times: at the 'start point' of the research, and then 6 and 12 months later in ENT Department. The videostroboscopy (VSS) examinations of larynx demonstrated on range of voice, symmetricity and amplitude of vocal fold's vibrations, mobility and closure of vocal folds. The maximal phonation time (MPT) and acoustic parameters such as: average fundamental frequency (Fo), Jitter, Shimmer as well as noise-to-harmonic ratio (NHR) were discovered. Manometric examinations of pharyngeal segment were carried out by using the esophageal ballon-based method with 4 balloon transducers. The following manometric parameters were analysed: the maximal contractions of the base of tongue (CBT), the UES resting pressure (RP); hypopharyngeal suction pump (HSP) as well as the oropharyngeal, pharyngeal and hypopharyngeal transit time (OTT, PTT, HTT, respectively) and velocity for bolus.

Results: Significant weakness of CBT ($p = 0.007$), decrease of HSP ($p = 0.006$) and decrease of velocity of bolus transit ($p = 0.001$) were particularly marked between the first and the third examination. MPT was shorter ($p < 0.001$) in women during all three examinations. The amplitude of vocal fold movement was shortened in all tests ($p < 0.001$), vocal

fold vibration was irregular in all patients during the third test ($p < 0.001$) and we observed incomplete vocal fold closure in all subjects in second and third examinations. Jitter and Shimmer increased ($p < 0.001$) especially in women. The value of vFo did not differ whereas NHR parameter was significantly higher in three tests, but only in women.

Discussion: This study shows that change on VSS (especially amplitude of movement, vibration and closure of vocal fold) was the most frequent observed in bulbar ALS cases. The voices of these patients were characterized with abnormal parameters of acoustic analysis and very short MPT. The results obtained from manometric examinations confirm progression of swallowing disorders.

Conclusion: The manometric and phoniatric examination are useful and supportive methods in the analysis of voice and swallowing disturbances in ALS patients.

P15 THE IMPACT OF PERCUTANEOUS ENDOSCOPIC GASTROSTOMY ON SURVIVAL IN PATIENTS WITH MOTOR NEURONE DISEASE: RESULTS FROM AN AUSTRALIAN MND SERVICE

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Keywords: PEG, area of onset, survival

Background: Up to 80% of patients with Motor Neurone Disease (MND) will develop swallowing difficulties. This can lead to dehydration, malnutrition and aspiration pneumonia. The option of percutaneous endoscopic gastrostomy (PEG) is often presented with the intention of providing a safer and more comfortable means of nutrition and hydration, improving quality of life and prolonging survival. However, the impact of PEG on survival continues to be debated.

Though there have been no randomised controlled trials (RCTs) looking at the impact of PEG on survival or quality of life, the evidence available shows a trend towards positive outcomes in both of these areas. Furthermore, there has been little research comparing the survival impact of PEG in bulbar- versus limb-onset MND.

Objectives: Our main aim was to compare survival of patients with and without PEG, suffering from bulbar- versus limb-onset MND.

Methods: A retrospective review of all deceased patients in the St Joseph's Hospital Multidisciplinary MND Service database from July 2006 to April 2011 was conducted. Sixty-six subjects were classified into four groups: bulbar-onset with PEG (n = 25); bulbar-onset without PEG (n = 5); limb-onset with PEG (n = 13); and limb-onset without PEG (n = 20). Survival times from the date of diagnosis until death were then compared.

Results: For bulbar-onset patients, those without PEG survived an average of 10 months post-diagnosis (range 1.5 – 48 months), compared to those with PEG who survived an average of 15 months post-diagnosis (range 4 – 32 months). Interestingly, we found the opposite in limb-onset patients, where those without a PEG survived an average of 23 months (range 1.5 – 72 months), while those with PEG survived on average only 18 months post-diagnosis (range 3 – 36 months).

Discussion and conclusions: A retrospective review of 66 deceased patients with MND showed an average five month survival advantage for bulbar-onset patients with PEG, compared to those without PEG. This outcome was predicted, as the presence of a PEG enables nutrition and hydration to be maintained until there is onset of respiratory failure.

For patients with limb-onset MND the opposite was found, with an average five month survival disadvantage in those with PEG. Anecdotal analysis of the limb-onset patients suggested factors contributing to a poorer outcome include delayed patient acceptance of PEG insertion and earlier onset and more aggressive progression of dysphagia in those who underwent PEG.

Larger studies comparing PEG survival advantages among the various types of MND are required. It would be beneficial if these studies included information regarding time of onset and severity of dysphagia for all patients. Furthermore, research into the reasons for delayed patient acceptance of PEG, as well as the effect of PEG on quality of life would be valuable.

P16 RELEVANCE OF NUTRITION ON SURVIVAL OF PATIENTS WITH MOTOR NEURONE DISEASE

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Keywords: survival analysis, percutaneous endoscopic gastrostomy, nutritional assessment

Aims: To evaluate the effect of nutrition on survival of patients with Motor Neurone Disease (MND) and present the predictor variables for indications of nutritional therapy, percutaneous endoscopic gastrostomy (PEG).

Methods: It was a retrospective longitudinal cohort study, from 2000 to 2008, and the sample consisted of 128 patients with MND. Clinical, nutritional and respiratory variables were analysed. Analyses were conducted by adopting the survival as the dependent variable. The survival curve was evaluated by Kaplain-Meier. The variables that had a significance level of 20% ($p < 0.20$) were selected for the proportional regression model of Cox.

Results: One hundred and eleven patients underwent gastrostomy, and 59 limb onset (ALS) and 52 with bulbar onset (PBP). Malnutrition was present in 32% of the population before PEG, most frequently in patients with limb onset. The survival time after PEG was 10.5 months for patients with PBP and 16 months for ALS ($p < 0.05$). Variables associated with survival were: early indication in the PEG, for ALS and PBP; reduction of FVC% and BMI before PEG (hazard ratio of 0.254, $p = 0.007$) for patients with limb onset and exclusion of oral feeding and tracheostomy (hazard ratio of 0.345, $p = 0.014$) for patients with bulbar onset.

Conclusions: Early insertion of percutaneous endoscopic gastrostomy, from the time of diagnosis was a protective factor for patient survival. Malnutrition was a bad prognostic factor, especially for patients with limb onset. Nutritional surveillance for disease progression may improve results when the goal is to increase the survival of patients with MND/ALS.

P17 SELF-ASSESSMENT OF THE DAILY FOOD INTAKE IN ALS VIA AN APPLICATION ON A MOBILE DEVICE

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Keywords: self-assessment, food intake, nutrition

Background: In the course of the disease an undesirable weight loss is common in ALS patients. Nutritional status is an important prognostic factor for survival in ALS. The early detection of alterations in the food intake as well as changes in weight is essential for these patients. Commonly a nutrition consultation is performed after patients develop swallowing difficulties or suffer from weight loss. The nutritionist anamnestically determines a retrospective dietary protocol by conducting a standardized interview to evaluate the daily oral food intake and the daily energy imbalance.

Objectives: To examine the recording of the actual nutritional intake in ALS via a web-based application on a tablet computer.

Methods: In a prospective, controlled study, patients recorded the oral food intake via an Internet-based nutrition application by using a touchscreen tablet computer in their home care environment. The application requires internet connection to store the data on a server located in the virtual and physical secured environment of the research facility. Based on the established "quartered plate method" the web application shows different options of meals, portions and durations. Patients assess the portion and duration of every single meal according to a full plate compared to their normal food intake. During the inclusion visit the nutritionist calculates the individual mean daily food intake of every participant and estimates individual portions. The self-assessment should be done at three predetermined days per week over a period of three months.

Results: We already included 10 patients. Every patient is provided with a tablet computer with the installed application and a nutrition consultation was performed. The intuitive user interface and the simple usability improve the compliance especially in patients with manual deficits.

Discussion: Web applications on tablet computers or on smartphones are well known by a wide range of internet users. To our knowledge we present the first web application for measuring the daily caloric intake in ALS. The study establishes the methodical feasibility and clinical tolerability of a web application for monitoring the daily food intake in ALS patients. Based on this technique, patients are able to record the nutritional intake between the outpatient visits; we suggest that use in clinical practice enables the early detection of changes for nutritional intervention.

P18 INTERNET-BASED SELF-ASSESSMENT OF APPETITE IN ALS

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Keywords: self-assessment, loss of appetite, nutrition

Background: An undesirable loss of weight over the course of the disease is common in ALS patients. Underlying causes are malnutrition, hypermetabolism, cachexia as well as a multifactorial reduction of appetite. The established ALS Functional Rating Scale, ALS FRS-R, records symptoms of dysphagia and manual deficits upon intake of food. The loss of appetite as a therapeutically relevant cause of undesirable weight loss is not reflected in the ALS FRS-R.

Objectives: In the context of a controlled study, we examined for the first time the symptom of loss of appetite in ALS by means of a specific self-assessment score.

Methods: In a prospective, controlled and stratified study, patients conducted a symptom-oriented self-assessment of appetite. An SQL database structure was developed for the technical realisation. Via encoded connections, a questionnaire is generated via the front-end of a web server featuring a specially secured operating system. Patients have access to a protected online portal via the URL www.ALShome.de. The internet-based self-assessment is realised by using the Council on Nutrition Appetite Questionnaire (CNAQ). This self-assessment questionnaire comprises 8 questions. Patients answer by using a 5 point scale. The score is a sum of these 8 items with a maximum of 40 points. Lower scores indicate deterioration in appetite, if the patient scores 28 or less a predicted weight loss of at least 5% within the next six months occurs. Besides the assessment of appetite we looked for an association between ALS related symptoms and a reduction of appetite.

Results: Fifty seven patients, 38 (67%) males and 19 (33%) females were included in this self-assessment trial. During the first self-rating 53% obtained the critical score. Patients with dyspnea more often displayed a critical loss of appetite, we detected 29 patients suffering from mild to severe dyspnea, of these 58.6% displayed the critical CNAQ Score, whereas only 31.8% of 22 asymptomatic patients were affected. The mean CNAQ Score in patients with dyspnea was 26.9, in patients without dyspnea 29.7 points.

Discussion: In our present internet based self-assessment study we first investigated loss of appetite in ALS patients. The collected data suggest that already 50% of the patients suffer from severe loss of appetite. Early ascertainment of indications for nutrition management including ecotrophological consultation, supplementary nutrition and pharmacotherapy represent essential objectives in the treatment of undesirable weight loss.

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THEME 2 MULTIDISCIPLINARY CARE AND QUALITY OF LIFE

P19 NFC BASED SELF-ASSESSMENT OF SYMPTOMS IN ALS

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Keywords: electronic self-assessment, near field communication, ALSFRSr

Background: With the development of new technical solutions, electronic capturing methods of symptoms in chronic conditions like ALS gains importance. In this respect self-assessment is a useful approach to include the patient's perspective in estimation of disease progression. Therefore we developed a smart poster that uses near field communication (NFC) technology. It forms, in combination with the ALS functional rating scale (revised) as a well evaluated and established self-assessment questionnaire, an easily applicable tool for the collection of health related information.

Objectives: To develop new PC independent elctronic methods for self-assessment in ALS.

Method: In a prospective controlled study 18 patients conducted a weekly self-assessment over a period of 12 weeks with the smart poster. The smart poster is based on radio frequency identification (RFID) as a form of NFC technology with very short range that enables data transmission between devices without confirmation. The single items of the ALSFRSr are arranged in a certain layout on the poster. A RFID chip with a distinct label is located behind every item of the score. With a NFC capable mobile phone the patient fills in the questionnaire without pushing any button, just by touching the answers with the phone. The phone reads, saves and sends the data to a server for visualisation on a web frontend.

Results: Eighteen patients (11 male, 7 female; 54 years on average) were included in the study. The smart poster and a suitable mobile phone were provided. There were no transmission errors or missing data. Causes for dropout were death (n = 2) and discontinuation without giving any reasons (n = 3). The baseline ALSFRSr on average was 33.7 the first ALSFRSr value captured with the smart poster was 33.3 (correlation 0.91, $p < 0.001$). After 12 weeks the ALSFRSr was 29.8.

Discussion: The electronic documentation of self-assessment in the home care environment could be seen as an addition to present paper based methods. The smart poster is a simple, easily applied, safe and PC independent way of gathering self-assessment data. This study proves the concept and supports the trend of comfortable data capturing through mobile devices in home care and clinical research.

P20 MND AWARE: TARGETED WEB-BASED AWARENESS TRAINING PROGRAM ABOUT MOTOR NEURONE DISEASE

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Keywords: online, training, health and community care professionals

Background: Health and community care professionals involved in the care of a person with motor neurone disease (MND) may have little or no knowledge about MND and the needs of people living with this disease. MND NSW delivers face-to-face education about MND but it is not always possible to deliver this immediately and/or at a convenient location.

Objectives: To increase health and community care professional understanding about MND and the needs of people living with MND through the development of web-based training about: MND awareness - MND and the impact it has on an individual's life; MND case management - effective ways of responding to people with MND.

Methods: A 26 question online survey was developed to elicit stakeholder MND experience and training and information needs. Learning objectives and a module structure were developed. The MND Aware training program was published online using a low-cost 'non-technical' commercially available e-learning authoring tool.

Results: 118 health and community care professionals from general community service, acute care hospital, non-government organisation, palliative care, rehabilitation, aged care, residential care and private practice work settings completed the online survey. 91% (n = 107) were from New South Wales, 9% (n = 11) from other Australia and New Zealand. The majority (69%, n = 79) had worked more than 2 years in a role with MND clients. All but one respondent wanted to be moderately informed (25%, n = 29) or extremely informed (74%, n = 86) about MND. The top 3 'things' respondents identified a health and community care professional should know about the needs of people living with MND related to approach to client management (28%, n = 91 mentions), strategies for symptom management and wellbeing needs (23%, n = 75 mentions), services available/access (17%, n = 55 mentions), disease types and progression (12%, n = 41 mentions), establishing client need (8%, n = 28 mentions), impact of MND (7.5%, n = 24 mentions) and training and support for care workers (1.6%, n = 5 mentions). From these findings learning objectives and the content was developed. Evidence-based content for each module was sourced. People with MND, carers and health and community care professionals were invited to contribute text, visual, audio and short video content.

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Discussion: Using stakeholder identified topic areas for MND training program content provides greater potential for the online training to be relevant to stakeholder needs. These topic areas included: approach to client management, establishing client need, MND disease types and progression, strategies for symptom management and wellbeing, services available/access, the impact of MND and training and support available for care workers.

Conclusion: Health and community care professionals have a keen interest in becoming more informed about MND, and have identified key topic areas for MND Aware module content. Online delivery provides ease of access over a large geographical area.

P21 PALLIATIVE CARE FOR PEOPLE WITH MOTOR NEURONE DISEASE: HOW EFFECTIVE IS AN EDUCATIONAL PROGRAM FOR SERVICE PROVIDERS?

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Keywords: palliative care, end-of-life, education

Background: Despite a recognised need for a palliative approach to caring for people with motor neurone disease (MND), access to palliative care is often limited and delayed. Education programs for health professionals are recommended to improve knowledge about MND and the integration of a palliative approach in MND care, however there is a lack of available programs to fill this identified gap.

Objectives: This project aimed to improve the knowledge of health professionals about a palliative approach to MND care through the development, implementation and evaluation of an educational program focused on the palliative care needs of people with MND.

Methods: A three-phase study was undertaken after initial consultations with service providers, carers and patients. Knowledge of palliative care and attitudes to providing MND care were measured pre and post delivery of the educational program and one month later via questionnaires. Interviews were conducted six months after the educational program. Non-parametric statistics were used to measure changes in knowledge and attitudes. Content analysis was used to investigate participants' experience of the program and impact on practice.

Results: The educational program consisted of 6 learning modules. Six one day workshops were held in WA and SA and 78 health professionals participated. Participants demonstrated improvement in MND and palliative care knowledge and attitudes which were maintained at the six month follow-up. Participants indicated that the gained knowledge positively influenced their clinical practice.

Discussion and conclusions: A targeted education program improved understanding about end of life care for people with MND, including understanding of physical and psychosocial needs of MND patients, ability to promote dignity and quality of life and better communication with MND patients. Currently, an implementation plan is being developed between the peak bodies of palliative care and MND to ensure widespread uptake of the educational program across Australia.

P22 INTAKE AND CASE MANAGEMENT OF PEOPLE LIVING WITH MND: HEALTH PROFESSIONAL AND COMMUNITY CARE WORKER EXPERIENCE AND VIEWS

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Keywords: case management, intake, health and community care professionals

Background: Timely response to identified needs, access to a coordinated and integrated care plan, regular monitoring and review of the person's condition, and appropriateness of the care plan are key aspects of the Statement of good practice for the management of ALS/MND (International Alliance of ALS/MND Associations 2007).

Understanding health professional and community care worker experience in and views about ALS/MND intake and case management provides insight into the training and information needs of this group.

Objectives: To identify health professional and community care worker experience in and views about ALS/MND intake and case management.

Methods: A 26 question online survey was developed to elicit stakeholder: experience in working with people living with MND; views about, issues emerging and strategies for managing MND intake and case management.

Issues emerging and useful strategies for intake and case management of MND clients are illustrated in 'text clouds' to allow the visualisation of word frequencies in text (www.tagcrowd.com/blog/about/).

Results: 118 health and community care professionals from community service, acute care hospital, non-government organisation, palliative care, rehabilitation, aged care, residential care and private practice work settings completed the online survey. 91%, (n = 107) were from NSW, 9% (n = 11) from other Australia and New Zealand. 69% (n = 79) had worked more than 2 years in a role with MND clients, 16% (n = 19) between 6 months and 2 years, and 9% (n = 10) less than 6 months (1 non-response). Just under three-quarters of all respondents (74%, n = 84) rated other health and community care professionals slightly informed or somewhat informed about MND, with a similar proportion (74%, n = 86) rating themselves as somewhat or moderately informed. All but one respondent wanted to be moderately informed (25%, n = 29) or extremely informed (74%, n = 86) about MND.

Of all respondents (n = 109, 9 non-responses), 28% (n = 31) do MND case management as a formal part of their role, 32%, n = 25 informally as part of their role and 39% (n = 43) do no MND case management. Of all respondents (n = 111, 9 non-responses), 24% (n = 27) do MND intake as a formal part of their role, 12% (n = 14) do MND intake informally as part of their role and 63% (n = 70) do no intake.

Discussion and conclusions: Health and community care professionals want to be more informed about MND. The identification of views about issues emerging and strategies for managing MND intake and case management provides

insight into the views and experiences of professionals in these roles. Using 'text clouds' to communicate these responses provides a visual 'at-a-glance' approach to understanding respondent experience, perceptions and strategies. This understanding provides a basis for developing information and training programs that address the current perceived needs of health and community care professionals as identified by them.

P23 NECESSITY OF MORE COORDINATOR NURSES FOR PATIENTS WITH ALS - A REVIEW ON A COORDINATOR NURSE'S ACTIVITY IN 5 YEARS IN A RURAL PREFECTURE

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Keywords: coordinator, nurse

Background: There is only one coordinator nurse working in the prefecture (population 1.87 million) in Japan, who has been accepting consultations for patients with intractable diseases (mostly neurodegenerative disease and especially focusing on ALS).

Objectives: We aimed to know the situation of the coordinator's activity and get some tips to better support patients with ALS in the prefecture.

Methods: We reviewed all the case records of the support for patients with ALS in the prefecture from 1 April 2006 to 31 March 2011.

Results: In a total of 3,123 records in the 5 years, there were 2,014 supports (64%) for ALS patients or families. The case records which had patients' details numbered 1,985 supports (for men 225, for women 760 times). The number of the patients on the records was 191 (age 30~86, 64.4 + /- 9.6 years old (mean + /- SD)). The upper 25 percentile was 48 patients, who accounted for 1,374 supports (69,2%). The distribution of supports showed a power series curve. Analysis on the 25 percentiles cases revealed that the factor to elevate number of supports was not age, sex, accepting a respirator, type of respirator (NIV or TIV), total ALSFRS-R score, respiratory function score, respiratory failure score, swallowing function score, or type of carer, but familial internal conflict ($p = 0.04$). Most support was conducted in neighbouring areas of the coordinator nurse's office.

Discussion and conclusions: The supports from the coordinator until now seemed to have focused on cases with familial internal conflicts and in relatively close areas of the coordinator. This suggested a lack of coordinator nurses and other supporting staff for the patients, like public health nurses, clinical psychotherapists, or social workers. We need to get more coordinator nurses for patients with ALS in each area.

P24 MOTOR NEURONE DISEASE: DISABILITY PROFILE AND SERVICE NEEDS AND GAPS IN AN AUSTRALIAN COHORT

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Keywords: disability, rehabilitation, health service utilization

Objective: Motor neurone disease (MND) places a substantial burden upon patients and their caregivers. This is the first study describing the disability profile and health-care needs for persons with MND in an Australian sample from the perspective of the patients and caregivers to identify current gaps in knowledge and service provision.

Methods: A prospective cross sectional community survey of persons with MND ($n = 44$) and their caregivers ($n = 37$) was conducted to determine symptoms and problems affecting their daily living. Standardized assessments were used to determine disease severity for stratification purposes; and service needs and gaps.

Results: The mean age was 61 with more men affected (3:2). Severity of disease was high ($n = 18$, 41% had severe disease) based on the Amyotrophic Lateral Sclerosis Functional Rating Scale. Despite the high level of disability, 11 (25%) solely relied on their families for all assistance. Caregivers were mostly partners (mean age 57). Persons with MND reported more pain, emotional disturbance and spasticity/cramps/spasms whilst caregivers focused more on psychosocial issues. 19 (43%) of persons with MND reported gaps in service in rehabilitation therapy and respite. Significantly proportionally more caregivers ($n = 19$, 51%) reported gaps particularly in the area of psychosocial support (formal paid care for personal assistance, additional carer support such as assistance with housework, respite care).

Conclusions: The gaps in MND care identified should be prioritised for future service development using the "neuropalliative rehabilitation" model of care. For improved consensus of care and communication amongst treating clinicians, the framework of International Classification of Functioning, Disability and Health should be explored in this population.

P25 TIMELY ACCESS TO PALLIATIVE CARE – IS THE DOOR OPEN?

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Keywords: palliative care, widening access, Foot in the Door project

Background: As a part of the UK government initiative on widening access to palliative care services for patients with non-cancer diagnoses, UK Hospice services are encouraging referrals of patients with progressive neurological disease.

Patients with MND are often referred late in their illness, when communication is difficult or lost and carers are exhausted. Our regional MND Care Centres offer palliative care input to all patients attending MND clinics and our local MND Association branch highlights hospice services. Yet there appears to be fear and reluctance to accept referral to hospice services until very late.

We obtained a grant from the Department of Health to develop an information and support programme for patients and carers not accessing hospice services, in partnership with the MND Assocaition and two regional MND Care Centres - at Barts and the London Hospital and the National Hospital for Neurology and Neurosurgery. We hoped to provide a non-threatening introduction to our services so that early referral would be welcome, not feared or postponed.

Objectives: To introduce MND patients, and their carers, to local hospice services at an earlier stage in their illness; to decrease anxiety and stress associated with referral to hospice services; to provide information on local community services; to provide opportunity to meet others with MND in a supported environment; to give opportunity to discuss future care options and planning; to offer support and information to carers and build their trust.

Methods: 41 patients and their carers were invited by letter and explanatory leaflet to a 10 week programme of information sessions including Breathing, Nutrition, Mobility, Communication, Future Choices and Carer Support at St Francis Hospice. Free transport was offered and a sitting service to enable attendance at carer sessions. Initially 7 patients accepted places but only 4 patients and 3 carers attended. A questionnaire using a Likert scale to assess effectiveness of this intervention before first session and at final session was completed.

Results: Evaluation of the questionnaires showed that all attendees gained more confidence in local services, had less anxiety about hospice support and felt more empowered to make future care choices. They also gave very positive verbal feedback. Since the programme, all patients have accepted input from hospice services.

Conclusion: Offering a structured programme of information and carer support sessions at an earlier stage in MND may provide a more acceptable introduction to hospice services than referral when needs are high. Our sessions at St Francis Hospice Day Centre confronted, but ultimately reduced anxiety about hospice services, providing a useful, early 'Foot in the Door'. Poor uptake of places needs further research.

A DVD was made to disseminate information on the programme and to capture the positive experiences of participants and encourage others to attend.

P26 LETTER ON FUTURE CARE; AN INDIVIDUALISED DISEASE-SPECIFIC FUTURE CARE PLAN FOR PEOPLE WITH ALS/MND

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Keywords: advance care directives

Background: Much attention has been given internationally to advance care directives to assist people in planning their future medical care.

An individualised letter expressing patient's wishes for their future care was offered to people with MND from 2001 to 2008. All patients attended a multidisciplinary MND service where they were seen by a palliative care physician. Each letter was drafted and revised over several consultations.

The letter conformed with health care guidelines on advance care planning. The original purpose of the letter was to prevent presentation to the emergency department during the terminal phase. It rapidly developed into a tool to facilitate planning and became known as the Letter on Future Care (LFC).

A series of studies was proposed to evaluate the LFC, focusing initially on the carers' experience.

Objectives: To test impact and acceptability of the LFC to patients and their carers. To obtain opinions of health care providers on practical issues governing use of the LFC. To develop guidelines for implementation of the LFC.

Methods: Past carers of MND patients were identified through the hospital record: 10 past carers of patients where a LFC was written and 9 where there was no LFC. Semi-structured interviews were held with each carer. Carers were shown a sample LFC. Interviews were analysed using qualitative research techniques.

A questionnaire was developed to elicit a critique of the LFC from a purposive sample of MND health professionals including neurologists, palliative care physicians and nurse specialists. Data were analysed using quantitative and qualitative techniques.

An expert panel was convened to develop the guidelines.

Results: Both groups of carers were generally very supportive of the LFC. Seven principal themes were extracted relating to benefits and suggested changes to the LFC emphasizing the value of the LFC for facilitating acceptance, allowing preparation and promoting autonomy.

Of 81 respondents to the health professional questionnaire, 69 (85%) agreed or strongly agreed that that the LFC would improve patient care.

The expert panel drafted practical and ethical guidelines using carer and health professional feedback.

Discussion: Carers found drafting the LFC empowering and liberating. The final document was valued as tangible evidence of the decisions made during discussions and an assurance that their wishes would be followed.

Health care providers supported the approach. Some critiqued the document for omitting detail of specific treatment and spiritual instruction. There was consensus that the process of drafting the LFC should be undertaken by the health professional who knew the patient best.

Prospective testing of the guidelines is planned.

Conclusions: Advance directives may be most useful when they are proposed by a trusted health professional and developed in cooperation with carers. The best results are individualised and address foreseeable disease-specific events.

P27 A NARRATIVE ANALYSIS OF STORIES OF DYING WITH MOTOR NEURONE DISEASE

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Keywords: narratives, dying, relatives

Background: There are diverse and conflicting discourses on dying with MND. The dominant discourses are those of the media - a terrible death involving choking and starvation; and the medical community - a peaceful death. To date constructions of dying with MND from the perspective of relatives has received little attention.

Objectives: To explore the experience of dying with MND from the constructions of the relatives of individuals who died with MND.

Methods: A qualitative study using narrative interviews was used to elicit the constructions of dying with MND. The data were derived from the narratives of twenty-one bereaved relatives. A combined thematic, structural and performative analysis of these stories was conducted.

Results: These narratives, characterised by plurality and diversity, revealed no accounts of choking or suffocation at the time of death, although one participant recounted a narrative of a painful death. While almost all of the narratives related accounts of dying quickly, peacefully and without pain, they were interwoven with experiences of suffering that occurred during the long trajectories of dying related by these research participants. Suffering was theorised as being both physical and iatrogenic in origin, related to the intermeshed components of the physical manifestations of MND and to the systems of health care, and the individuals within this system, upon which the dying person and his or her family were dependent.

Conclusion: This study contributed to existing knowledge by focusing on relatives' narratives of dying which revealed detailed constructions in which dying with MND was considered to encompass the entire disease trajectory. The purpose of this poster is to present the findings of the study. Brief extracts from four of the stories from this data set are used to illustrate different constructions of dying with MND. They highlight the diversity of experiences recounted by the narrators.

P28 A RANDOMISED CONTROLLED TRIAL ON THE EFFECT OF CASE MANAGEMENT ON QUALITY OF CARE, QUALITY OF LIFE AND CAREGIVER BURDEN IN ALS

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Keywords: quality of life, case management, quality of care

Background: The aim of palliative care in patients with ALS and their primary caregivers is to maintain patients' health related quality of life (QoL) and to decrease caregiver burden. Proactive, multidisciplinary care is focused on supporting bereavement, symptom control management, decreasing activity limitations and participation restrictions of the patient and on support of the emotional and physical strain of the caregivers. From clinical practice and patients' and caregivers' quality of care evaluations we know that palliative care does not always meet the needs of patients with ALS and their caregivers. Additional case management, independent from regular care providers, may improve quality of life for patients with ALS and may reduce caregiver burden.

Objectives: The primary objective was to investigate whether case management improves QoL of patients with ALS. Secondary objective was to evaluate the effect of case management on the caregivers' burden.

Methods: Participating ALS patients and their caregivers were randomized in either usual care (provided by a specialized multidisciplinary ALS team) plus case management or usual care alone. During 12 months, case management was provided by 2 occupational therapists who guided patients and caregivers through a client centred approach. They visited the patients and caregivers at home at study entry and every three months. Quality of life (the 40 item ALS Assessment Questionnaire/ALSAQ-40) and caregiver burden (Caregiver Strain Index) was assessed by an independent examiner at study entry and after 4, 8 and 12 months. We used generalized estimating equations (GEE) to examine the impact of case management on changes in ALSAQ-40 and CSI over 1 year.

Results: From March 2009 to July 2011, 132 patients with ALS and their caregivers from all regions of the Netherlands participated in our study. The mean age of the patients was 63 (+ 11) years and the mean score on the revised Amyotrophic Lateral Sclerosis Functional Rating Scale was 32 (+ 8) points. Twenty-four percent of the patients had bulbar onset. Seventy-one patients and 66 caregivers participated in the intervention group, 61 patients and 60 caregivers in the control group. Groups were comparable on demographic and clinical characteristics. Preliminary results from a subset of the participants (89 patients with ALS and 86 caregivers) showed no effect of case management on changes in 1 year in quality of life and caregiver strain. In both groups, patients' ALSAQ-40 total scores increased (reduced quality of life) by 25.2 (SE 2.1) points/year and by 3.8 (SE 1.8) points/year for the ALSAQ-40 domain score Emotional Functioning. In both groups, caregiver strain increased by 2.4 points/year (SE 0.3).

Discussion and conclusions: Our preliminary analysis indicates that case management for patients with ALS and their

caregivers does not improve QoL of the patients and does not reduce caregiver burden.

P29 PATIENTS' AND CAREGIVERS' PERSPECTIVE ON CASE MANAGEMENT IN ALS CARE

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Keywords: case management, quality of care, qualitative study

Background: The concept of case management has been suggested as an innovative strategy to optimize complex multidisciplinary care in patients with ALS and their caregivers. However, high quality evidence on the effectiveness of case management in ALS care is not yet available. Our ongoing cluster-randomized controlled trial (RCT) on case management in patients with ALS and their caregivers revealed that the extent to which patients and caregivers rely on case management varies considerably (1). Experiences of patients and caregivers with case management and their needs for case management provide insight in preferences for care that may facilitate implementation of case management services for patients with ALS.

Objective: To explore the experiences of patients with ALS and their caregivers with case management.

Programme description: Our qualitative study was nested in a large cluster-randomized controlled trial on the effectiveness of case management on quality of life of patients with ALS and their caregivers. We provided case management to 71 of the 132 included patients in addition to usual care from the multidisciplinary ALS care team. We undertook in-depth, narrative interviews with ALS patients and their primary caregivers who received case management. Interviews were held at the end of the intervention phase of the RCT, i.e. 12 months after baseline assessment.

All interviews were audio-recorded, transcribed and checked for accuracy of transcription. Content analysis methodology was used to initially organize data and subsequently to identify the recurrent themes.

Clinical outcomes: Ten patients with ALS (7 male, mean age; 60.8 years, mean ALS-FRS-R score; 34.9) and their primary caregivers participated in our qualitative study. Interview duration ranged from 60-90 minutes. Three recurrent themes emerged: 1) offering emotional support, 2) professional expertise in ALS care and 3) providing practical support. Home visits and taking the time to sit with participants were highly appreciated. Higher needs for case management appeared to be associated with a limited social network, dissatisfaction with the ALS team, not daring to ask for help, a rapid disease course, the timing of the case management period in the disease course and a limited empowerment of participants.

Recommendations to the field: For patients with ALS and their caregivers, case management can be a valuable adjunct to ALS care. Patient and caregiver input should be included in implementing case management services in complex multidisciplinary ALS care.

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P30 THE IMPORTANCE OF DIRECT-CARE HOME ASSISTANCE FOR PEOPLE WITH AMYOTROPHIC LATERAL SCLEROSIS AND THEIR CAREGIVERS: A LONGITUDINAL STUDY

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Keywords: quality of life, direct-care home assistance, psychological well-being

Background: The importance of home assistance in Amyotrophic Lateral Sclerosis (ALS) is evident, but so far, no studies have investigated with a scientific approach the real impact on psychological well-being of patients and caregivers.

Aims: We aim to evaluate if the presence of a direct-care worker improves ALS patients' and their caregivers' quality of life and well-being.

Methods: 40 ALS patients, together with their primary caregivers (a close relative living with patient), completed Beck Depression Inventory (BDI), McGill QOL Questionnaire (MQOL) and State-Trait Anxiety Inventory (STAI); caregivers also filled Zarit Burden Inventory (ZBI) and patients were assessed with ALSFRS. The presence of a direct-care home worker was analyzed. Subjects were followed during the period of one year, assessed every 4 months.

Several statistical analyses were conducted.

Results: Fifteen patient-caregivers couples (37.5%) indicated the presence of a direct-care worker. Comparing subjects with and without home assistance, we found no statistical differences between groups for psychological variables, but ALSFRS was higher in patients with assistance. We conducted MANCOVAs, using ALSFRS as covariate and psychological scores as dependent variables, comparing the two groups. Weighting the effect of ALSFRS, all patients' and caregivers' psychological variables were significantly different during time. Subjects with direct-care assistance reported higher scores in MQOL and lower in BDI and STAI and caregivers' scores of ZBI were lower. Furthermore, the introduction of a direct-care worker in families where it was not present, seems to improve significantly (p from < 0.05 to < 0.01) caregivers' and patients' psychological well-being.

Conclusion: With the influence of patient's loss of functions in data analysis weighted, our data indicate that the presence of a direct-care home worker has a positive effect on psychological well-being of ALS patients and their caregivers,

improving their quality of life and reducing anxiety, depression and care burden.

P31 ASSESSMENT OF BURDEN OF THE CAREGIVERS OF PATIENTS WITH ALS USING ZARIT BURDEN INTERVIEW (ZBI): STUDY OF CORRELATIONS WITH FUNCTIONALITY, QOL, MOODS DISORDERS AND TECHNICAL AIDS

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Keywords: palliative care, caregiver burden, quality of life

Background: ALS is one of the most devastating neurological diseases in adults with unpredictable clinical course. Though many poor prognostic factors are well known, a few recent studies have also shown that the role of physical and emotional burden of the caregiver is an issue that must be addressed and should be part of management by a multidisciplinary team. Several other factors have been identified with low correlation level as predictors of QoL both in patients and caregivers. However, trial designs implemented in single ALS centers with small or low representative samples may have methodological flaws. Therefore, we aim to clarify these issues.

Objective: To evaluate caregiver burden and its impact on patient's quality of life.

Programme description: Observational study in two Portuguese regions (Northern and Southern country), of 39 consecutive caregivers/ALS patients.

Assessment: The two groups: G1 had 20 patients/caregivers from National Referral Center at Lisbon (Southern) and G2 (Northern- Oporto and Coimbra) had 19 patients/caregivers. To measure caregivers' burden we used the Zarit Burden Index (ZBI). Patients were evaluated with the ALSFRS, the EQ-5D, the HADS and a semi structured interview to evaluate issues regarding team members, palliative care and technical aids used to improve functionality. The interviews were carried out between March 2008 and April 2009. We tested the group homogeneity and analyzed their differences. To find out predictors of caregiver's burden and their impact on patients QoL, we performed univariate and multiple regression analysis weighted by the detected regional differences.

Clinical outcomes: Patients in G1 included: 15 males and 5 females; 16 spinal onset and 4 bulbar onset. Patients in G2 included: 15 males and 5 females; 15 spinal onset and 5 bulbar onset. Mean disease duration was 28 ± 27.7 months in G1 and 57 ± 58.3 months in G2, mean EuroQol-5D was 9.40 ± 1.8 (G1) and 9.95 ± 2.32 (G2), the average age was over 60 years for both groups, with no significant differences regarding socio-demographic and moods data. ALSFRS scores were higher in G1 (24.9 ± 8.66) vs G2 (18.25 ± 9.86) (t-test; $p = 0.028$). QoL showed a trend related to the amount of technical aids used ($p = 0.06$) and no other significant relationships to other variables including mood disorders in multiple regression. The caregivers' relationship to patients were predominantly spouses, mean ZBI was 48.75 in G1 and 52.42 in G2. There were significant regional differences regarding social embarrassment ($p = 0.014$), lost of social role ($p = 0.08$), and mood ($p = 0.027$). We found statistically significant correlations between ZBI and TA ($p = 0.05$), EQ-5D($p = 0.03$) and ALSFRS ($p = 0.019$).

Conclusions: This study demonstrated the close relationship that exists between the funcionality of patients with ALS, their QoL and mood disorders with the physical and emotional burden of caregivers. It is extremely important to pay attention and prioritise needs of caregivers/patients, in order to drive better care for this population with very special characteristics.

P32 THE REALITY OF ALS CAREGIVERS' USE OF SOCIAL RESOURCES AND POSITIVE PERCEPTION

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Keywords: home care, family caregiver, positive perception

Background: Researchers tend to focus on negative aspects such as care burden when discussing ALS caregiver's caregiving. Caregivers would have both positive and negative feelings on caregiving but their positive perception of caregiving for ALS patients has not been figured out.

Objectives: The purpose of this study is to examine the way care for ALS patients ought to be by clarifying the reality of caregivers' positive perceptions of caregiving for ALS patients on each stage of their use of public/non-public social resources.

Methods: We conducted a mail questionnaire survey of 1,000 caregivers who are members of Japan ALS Association and engage in home care now. Questions were composed of the Caregiving Gratification Scale (the range of the scale is 0-24 points)(1) for measuring positive perception, characteristics of patients and caregivers, and the use of public/private social resources. The severity of ALS patients showed mild to moderate-severe or severe in three stages, depending on the degree of disability. We conducted t-test and one-way analysis of variance, and calculated the Pearson product-moment correlation coefficient on each survey item.

Results: 496 questionnaires were returned (response rate 49.6%), among which 371 were valid (valid response rate 37.1%). The average score of the scale of satisfaction with care was 16.9 ± 4.6. We divided its score into three stages and found that the score of moderately-severe ALS patients (those who "require assistance in daily life," "have difficulty breathing/coughing up phlegm," and "have dysphagia") was 15.4 ± 4.6, whose score was statistically significantly lower than other stages of ALS patients. The analysis of moderately-severe ALS patients' positive perception and use of public/non-public social resources shows that the items of "the use of home help service" and "the number of days home nursing was used" were statistically significant.

Discussion and conclusions: Our research revealed that the score of positive perception among caregivers who care for moderately-severe ALS patients were the lowest. It also suggested that their use of home help service and home nursing contributed to enhance their positive perception of

caregiving. We consider it important for caregivers to introduce social resources appropriately by preparing for expected disease progression of ALS. Our study also showed that the score on a scale of ALS caregivers' satisfaction with care in our research was higher than that of caregivers in charge of "patients requiring long-term care" found in earlier studies. We need to conduct further research to examine whether only caregivers who care for ALS patients have a high positive perception of caregiving.

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P33 A SURVEY INVESTIGATING THE INTERVENTIONS AND OUTCOME MEASURES USED BY CHARTERED PHYSIOTHERAPISTS IN IRELAND IN THE MANAGEMENT OF PERSONS WITH MOTOR NEURONE DISEASE / AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: physiotherapy, survey, rehabilitation

Background:Varying levels of evidence exist regarding the work of physiotherapists in the care of persons with Motor Neurone Disease/Amyotrophic Lateral Sclerosis. Several international guidelines advise on the assessment and management of respiratory symptoms. Clinical trials and systematic reviews have investigated exercise prescription. Published physiotherapy reviews report on the management of pain and decreasing functional performance. A range of papers have commented on the use of a variety of outcome measurements.

Objectives: This study aims to identify the interventions and outcome measures used by Chartered Physiotherapists in Ireland in the management of persons with MND/ALS.

Methods: Chartered Physiotherapists were invited to complete an anonymous online survey investigating their recent practices with persons with MND/ALS. Responses were converted to an Excel Spreadsheet for analysis and presented as percentages.

Results: Oxygen saturation was the most used respiratory test as was used by 89.7% (26/29) of respondents. In treating respiratory symptoms, the majority of respondents addressed dyspnoea (42/44; 95.5%), secretion management (41/45; 91.1%) and education and training related to Non-Invasive Ventilation (29/44; 65.9%). Respondents identified several variables influencing prescription of exercise, with low intensity exercise being prescribed by most respondents (34/35; 97.1%). Respondents involved in the management of physical pain used a variety of interventions, with all respondents using positioning (45/45, 100%) and passive exercise (44/44, 100%). In addressing patients' deteriorating functional status, the majority of respondents advised regarding moving and handling (41/42; 97.6%). In measuring outcome, the majority of respondents use the Timed Up and Go (21/40, 52.5%).

Discussion and conclusions: The majority of respondents are using interventions in line with international guidelines in some areas of care (management of respiratory symptoms), but not all areas of care (assessment of respiratory status). In other domains, current research and guidelines are inconclusive (prescribing exercise, managing pain, addressing functional decline).

P34 MANAGING NECK PAIN AND OPTIMISING HEAD SUPPORT IN PEOPLE WITH MOTOR NEURONE DISEASE

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Keywords: neck and shoulder pain, head control, head supports

Background: Progressive muscle weakness is a characteristic of Motor Neurone Disease. Muscle weakness affects the limbs, and the neck and shoulder muscles that support the head. Weakness of neck and shoulder muscles can contribute to neck and shoulder pain, and cause problems with head positioning and control. The incidence and causes of neck pain and poor head control are not well documented; further, there is minimal literature about methods of optimising head support. Head control and positioning is vital for optimal respiratory function, communication and swallowing - poor control can have a significant negative impact on these and other ADL tasks.

We sought to explore the relationship between neck and shoulder pain, head support usage and satisfaction with head supports in patients treated at St Joseph's Hospital in the western suburbs of Sydney.

Objectives: The aims of the study were first, to review the incidence of neck and shoulder pain, and the characteristics of this pain, since symptom onset. Second, we sought information on the usage of head supports and their effectiveness. Third, we sought to describe the impact that both pain and poor head position have on ADL function. Our final goal was to identify the difficulties people encountered when using the most commonly prescribed head supports.

Methods: Patients currently on the MND register at St Joseph's Hospital were given a questionnaire and underwent a semi-structured interview to obtain information on neck pain, head support usage and difficulties and limitations with head supports.

Results: The results of the patient interviews will be presented. The results support the relatively high incidence of neck and shoulder pain in people with MND. Pain and poor head control had an adverse affect on ADL performance. Current head supports do provide some head control, but adversely affect swallowing, communication and breathing by immobilising the jaw. The feeling of claustrophobia caused by neck supports was also raised. Other identified issues will be discussed, and possible solutions for this issue that affects the quality of life for people with MND will be highlighted.

Discussions and conclusion: Neck and shoulder pain, and poor head control are issues that are common in people with MND. More needs to be done to develop better neck supports that allow adequate head support while not interfering with swallowing, communication and respiration. Knowledge of the information collected by this study will help all who treat people with MND better manage the symptoms of neck and shoulder pain, and the issues surrounding head control in people with MND.

P35 THE POWER OF TOUCH - AN EVALUATION OF A VOLUNTEER MASSAGE PROGRAM PROVIDING A HAND AND FOOT MASSAGE TO PEOPLE LIVING WITH MND

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Keywords: massage, volunteer, social connection

Background: Gentle massage has been recommended for people with MND to preserve range of motion; ease joint stiffness; and muscular tightness. Many people with MND report regular massages assist with relaxation and improved physical comfort.

During 2010, the MND Association of NSW (MND NSW) recruited and trained volunteers to equip them with the knowledge and skills to provide a simple hand and foot massage. Volunteers then were matched with people with MND to provide regular and ongoing massages.

MND NSW acknowledges the support of the MND Association of Victoria in sharing their experiences of developing a similar massage volunteer program.

Objectives: To evaluate the outcomes of providing a hand and foot massage service to people with MND; volunteers; and the organisation.

Methods: 12 massage recipients and carers were interviewed by phone or email. 10 volunteers were interviewed by phone and attended face to face de-briefing meetings to discuss their experiences. MND NSW staff were interviewed face to face.

Results: Qualitative information gained from the interviews indicates the massage volunteer program has specific benefits for recipients, volunteers and the organisation.

Recipients report temporary physical benefits that included easing of pain, discomfort and swelling as well as increased warmth in hands and feet. Other benefits included feeling calmer and more relaxed. Recipients also reported enjoying social contact with the volunteer as well as their visit giving their carer a break from their caring role. Whilst most received a weekly massage, those who didn't indicated, although grateful for a massage less often, their preference was to have a weekly massage.

Volunteers report providing the massage gives them a social connection with the recipient and an ability to contribute something practical. They have learnt useful massage skills and report enjoying the volunteer massage experience.

MND NSW staff report their connection with those referred to the massage program is enhanced through being able to monitor them regularly through feedback from the volunteers. They also report the additional social support is beneficial for carers. Whilst the organisation has support in place for volunteers to manage grief and loss issues arising, one unexpected issue raised is the impact of ongoing relationships between volunteers and people with slowly progressive MND.

Discussion: Massage cannot reverse or slow the progression of MND but there are benefits not only for people with MND, carers and volunteers providing the massage but also the organisation employing them.

Conclusions: The benefits of providing a volunteer massage service are clear. It enhances the community of care available to people living with MND and MND NSW is now looking at how the program can be developed into regional and rural areas through utilising volunteers employed by other community service providers.

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THEME 3 COGNITIVE AND PSYCHOLOGICAL ASSESSMENT AND SUPPORT

P36 THE ROLE OF FATIGUE AND PSYCHOSOCIAL PHENOMENA IN THE DETERMINATION OF QUALITY OF LIFE FOR PATIENTS WITH MOTOR NEURONE DISEASE

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Keywords: quality of life, fatigue, depression

Background: Quality of life (QoL) for patients with MND has been shown to be unrelated to functional impairment. Whilst some research has been undertaken to evaluate factors that impact upon patient quality of life, to date no research has evaluated the direct and indirect causal links between psychological factors in a structured model for MND.

Methods: One hundred and seven patients with MND completed a suite of questionnaires containing measures for fatigue, depression, anxiety, functional status, social withdrawal and quality of life. The fatigue, depression, anxiety, coping and social withdrawal scales were modified in order to satisfy the demands of the Rasch model on a separate sample of 298 patients with MND. Hypothesised causal relationships between the study variables were tested using structural equation modeling (SEM).

Results: The final model was shown to have excellent fit characteristics ($\chi^2(5) = 6.06$, $p = 0.30$; CFI = 0.99; GFI = 0.98; RMSEA = 0.045). Quality of life (QoL) was primarily driven by strong direct effects from depression ($\beta = -0.47$, $p < 0.001$) and social withdrawal ($\beta = -0.34$, $p < 0.001$) in addition to an indirect effect of coping ($\beta = 0.35$, $p < 0.001$), mediated primarily though anxiety ($\beta = -0.39$, $p < 0.001$) and depression ($\beta = -0.36$, $p < 0.001$). Fatigue exerted a strong direct relationship with anxiety, depression and social withdrawal ($p < 0.001$).

Discussion: This study highlights the importance of depressive symptomology and social withdrawal in the determination of patient QoL in MND. Coping was found to be a strong modifier of both depression and anxiety, and had a strong indirect effect upon QoL. Fatigue was shown to impact strongly upon anxiety, depression and social withdrawal although it did not elicit a significant direct effect upon QoL in this model.

P37 THE DEVELOPMENT AND VALIDATION OF A BRIEF SCREENING QUESTIONNAIRE FOR COGNITIVE IMPAIRMENT IN ALS

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Keywords: cognition, dementia, screening

Background: It is estimated that 25-50% of ALS patients develop cognitive impairment during the course of their illness. The clinical significance of this cognitive impairment is becoming increasingly apparent. Cognitive dysfunction in ALS patients has been linked to poor decision making, reduced compliance with medical interventions, and increased carer burden as well as shorter survival. Comprehensive neuropsychological assessments do not constitute a practical option in busy multidisciplinary clinics. There is an urgent need for a validated screening tool that can be used by clinicians in busy clinics to identify ALS patients with cognitive impairment.

Objective: To describe a brief cognitive questionnaire (the Brief Screening Questionnaire for ALS Patients, BSQ-ALS), based on detailed neuropsychological assessment of a population-based cohort of incident ALS patients.

Methods: The questionnaire was developed following detailed assessment of 160 incident patients with ALS. It takes 7-10 minutes and includes a brief assessment of executive, memory and language functions. Within three months of completing the questionnaire, patients undergo a comprehensive home-based neuropsychological and clinical assessment. Similar neuropsychological evaluations are undertaken in age, sex and education matched healthy controls.

Results: We present the rationale for the current design of the questionnaire. Evaluation of executive function includes three tasks including similarities, response inhibition and a three minute verbal fluency task. Several aspects of memory function are assessed including registration, recall and recognition. Language function evaluation includes noun and verb naming as well as tests of comprehension. We also present the proposed methodology for validation of the questionnaire using the formal neuropsychological battery as the golden standard. We also compare the clinical utility of the information obtained using the questionnaire to other cognitive questionnaires, and to data obtained using only the verbal fluency task only.

Conclusion: This study describes the clinical utility of a 10 minute cognitive screening questionnaire in a population based setting.

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P38 PRAGMATIC LANGUAGE FUNCTION IN MOTOR NEURONE DISEASE

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Keywords: pragmatics, language, cognition

Background: Previous research had documented that persons with Motor Neurons Disease (PwMND) experience changes in recognizing the affective states of others. What is less clear is the extent that other aspects of social interaction are impaired by the disease.

Objectives: The current research study aimed to examine the profile of pragmatic language function in a sample of PwMND and healthy controls. It was anticipated that changes in aspects of pragmatic language would be apparent in PwMND.

Methods: Pragmatic language function was assessed in 31 persons with MND and 15 healthy controls, via speech pathologist-rated video-taped conversations. Nominated caregivers also completed questionnaires rating PwMND's overall pragmatic language functioning. Data were analyzed using non-parametric methods.

Results: Overall, PwMND were rated as more impaired in their pragmatic language function than healthy controls ($p = 0.000$). This difference was stable across both clinician and caregiver ratings. A profile of pragmatic difficulties was apparent in the MND group. PwMND were rated as having more frequent difficulties with providing ongoing contributions that maintained the flow of conversation. Conversational quality was abnormal, with content often sparse or verbose. Word finding difficulties also affected pragmatic communication. PwMND also showed reduced variation of communication style across conversational contexts. Consistent with clinical expectations, PwMND were rated as impaired on conversational prosody, fluency and voice quality. However, this is to be expected given the physical symptomology associated with MND.

Conclusion: In PwMND, pragmatic language function was significantly impaired, relative to controls. The profile of abnormal pragmatic language, in addition to well-recognized deficits in affect recognition, many have significant implications for interpersonal relationships and even everyday social interactions. These findings highlight the importance of pragmatic language assessment in tracking the development of non-motor symptoms in MND. It is recognised that the physical changes in PwMND do contribute to changes in pragmatic communication. However, physical changes alone did not explain the range of changes observed in this sample.

P39 A NATIONAL ALS MULTICENTER STUDY FOR THE CHARACTERIZATION OF EMERGING COGNITIVE AND BEHAVIORAL DECLINE

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Keywords: frontotemporal disease, language, behavior

Background: Current formulations of frontotemporal disease posit a synergism between the bilateral frontotemporal cortical regions in the emergence and progression of disease.

Objectives: Our objective was to investigate the pattern of emergence of cognitive and behavioral decline in ALS in a national sample, which would allow us to evaluate potential differences between rural, suburban, and urban population samples. Given that cognitive decline in ALS is generally associated with frontotemporal disease, characterized by disorders of language or behavior, we also sought a sample size large and diverse enough to examine male: female differences in language and behavior disorder incidence rates.

Methods: Designated personnel from 14 ALS Multidisciplinary clinics were trained by AB in standardized administration of the Penn State Brief Exam of Frontal and Temporal Dysfunction Syndromes (PSFTS). All patients evaluated met El-Escorial criteria from definite to possible ALS. Exclusion criteria included CNS co-morbidities potentially affecting cognition. Following exam administration, data were downloaded in a central depository for access by the Penn State research team.

Results: To date, data of 100 subjects from rural ($N = 28$), suburban ($N = 60$) and urban ($N = 12$)regions have been collected, with rural and suburban data analyzed. Male ($N = 40$) and female ($N = 48$) subjects were equivalent in ALS FRS-R scores (32.8/29.6) and FVC percent of predicted (75.8/68.6). Significant differences were detected for age ($M = 56.5/61.7$) ($p = 0.037$) and education ($M = 15.9/14.5$) ($p = 0.008$) with the male sample somewhat younger and higher educated. Incidence rates of cognitive deficiency or behavioral change were found to be equivalent across rural and suburban regions: letter fluency (LF) (23.5%), category fluency (CF) (22.5%), reading comprehension (13.4%), 2-D constructions (CON) (30.2%), Frontal Behavioral Inventory (FBI) findings approaching (> 20) (15.7%) or exceeding (> 27) (4.5%) clinical significance. Despite lower education levels, females scored higher than males in CF ($p = 0.014$). Correlations were found for CF but not LF with respect to CON for the total ($p = 0.009$) group, for males ($p = 0.05$) and females ($p = 0.044$), and for the suburban ($p = 0.010$), but not the rural ($p = 0.402$) group.

Discussion and conclusions: The relationship between CF and CON lends support to the concept of a synergism between bilateral temporal cortical processing in lexical access for categorical information; with female gender potentially associated with delayed emergence of cognitive decline in the presence of temporal dysfunction. Findings were consistent with our recent validation study applying Guilford's Structure of Intellect Theory (1), as well as recent imaging evidence of a continuum of extra-motor cerebral and cognitive change in ALS related to cortical - subcortical neural network processes (2).

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P40 MND AND FTD: CONTINUUM OR OVERLAP?

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Background: Although previously considered as a pure motor disorder, it has become clear that a significant proportion of patients with motor neurone disease (MND) develop cognitive impairment and behavioural changes, which may be severe enough to meet criteria for frontotemporal dementia (FTD). Few studies have examined cognitive and behavioural changes in unselected cases and there have been no comparisons of MND with FTD.

Objectives: To compare the cognitive and behavioural profile of patients with MND versus those with behavioural variant FTD (bvFTD).

Methods: Twenty consecutive patients with MND, 20 with bvFTD and 20 healthy controls completed a neuropsychological assessment including cognitive screening (Addenbroke's Cognitive Examination Revised-ACE-R), working memory (Digit span), inhibitory control (Hayling test), decision making (Iowa Gambling Test- IGT) and evaluation of neuropsychiatric symptoms (Cambridge Behavioural Inventory CBI-R). Groups were matched by age and education.

Results: Nine of the 20 (45%) MND patients had marked cognitive impairment on testing and 5 of them met criteria for FTD. Controls performed better than both MND and bvFTD groups on the ACE-R as well as a wide range of other tasks. After excluding the 9 cases with frank cognitive impairment, the cases with pure MND had a worse performance than controls on the Hayling test of inhibitory control. Lack of motivation was the most prominent neuropsychiatric feature in MND, occurring in both those with and without cognitive impairment.

Discussion and conclusion: Significant heterogeneity exists in the cognitive and behavioural profiles of MND patients. MND patients with clear cognitive impairments presented a similar pattern to those with bvFTD. A subset of MND patients apparently cognitively intact, had a subtle impairment on control inhibition and a significant lack of motivation, reinforcing the idea of a clinical continuum between MND and FTD.

P41 NEUROPSYCHOLOGICAL PROFILE OF COGNITIVE CHANGE IN A SAMPLE OF SLOVENIAN ALS PATIENTS: THE RESULTS OF A PRELIMINARY STUDY

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Keywords: cognitive functions, executive functions, everyday life performance

Background: Traditionally, ALS has been viewed as a disease of the motor neuron system, characterized by degeneration of both upper and lower motor neurons, with no compromise to cognitive functions. Recent studies have shown that structural and pathological changes are not confined to motor areas and that these changes correlate with cognitive dysfunction. However, the nature and the extent of cognitive change in patients with ALS are not clearly defined yet.

Aim: The purpose of this study was to examine the profile and the extent of cognitive deficits in a sample of Slovenian ALS patients.

Participants and methods: A total of 22 non-demented ALS patients (median age = 59.5 years, interquartile range 51.7-64.0 years) and 21 age, sex and education matched healthy controls were compared on a comprehensive battery of neuropsychological tests of cognitive functions (executive, language, visuo-spatial functions and memory). A special emphasis was placed on executive functions. Standard neuropsychological tests were appropriately controlled for motor impairment in patients and all participants were evaluated also on ecologically valid motor-free test of cognitive functions. All patients were being treated at the ALS Centre at the Institute of Clinical Neurophysiology, University Clinical Centre Ljubljana, at the time of the inclusion into the study.

Results: The results show that participants with ALS are significantly impaired on tests of executive functions, language and memory but not on tests of visuo-spatial functions. The differences between ALS and control group are smaller after appropriately controlling for motor dysfunction, however, the robust differences still remain. According to Schulz criteria of cognitive dysfunction 15 patients (68%) can be classified as severely cognitively impaired (scores lower than 5th percentile on at least two measures in two different cognitive domains or on more than two measures within a single cognitive domain).

Conclusion: We conclude that the studied sample of ALS patients shows significant cognitive dysfunction. The results further show that motor dysfunction can present a significant confounding factor when using standard neuropsychological measures, but even when appropriately controlling for motor dysfunction, ALS patients show cognitive deficits, including those with possible effects on their everyday life performance. Further research work is needed to precisely map and confirm its exact nature and extent.

P42 NEUROPSYCHOLOGICAL STUDY OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS/PARKINSONISM-DEMENTIA COMPLEX OF THE KII PENINSULA OF JAPAN

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Keywords: parkinsonism-dementia complex, neuropsychological study, Kii

Background: The Kii peninsula of Japan, together with Guam, has been one of the highest incidence foci of amyotrophic lateral sclerosis and parkinsonism-dementia complex (ALS/PDC) in the world. The purpose of this study is to clarify neuropsychological features of patients with ALS/PDC of the Kii peninsula (Kii ALS/PDC) of Japan.

Methods: Fourteen patients with Kii ALS/PDC (ALS 4, PDC 8 and parkinsonism-dementia ALS: P-D-ALS 2), 12 patients with Alzheimer disease (AD), 10 patients with progressive supranuclear palsy (PSP), 10 patients with frontotemporal lobar degeneration (FTLD) and 10 patients with dementia with Lewy body (DLB) were subjected to history taking about clinical symptoms, brain MRI, SPECT, and neuropsychological tests. The neuropsychological tests consisted of Mini Mental State Examination (MMSE), Raven's Colored Progressive Matrices (RCPM), verbal fluency, paired associate word-learning test (PAWLT) and the Frontal Assessment Battery (FAB).

Results: Two patients with Kii ALS had no cognitive dysfunction, and the rest of the patients with Kii ALS/PDC had dementia. Brain MRI showed atrophy of the frontal and temporal lobes, and SPECT revealed a decrease in CBF of the frontal and temporal lobes in all patients with cognitive dysfunction. Disorientation, difficulty of serial 7's and 3 word recall of MMSE, delayed reaction time of RCPM, difficulty of word recall of PAWLT and low score of FAB were recognized in Kii ALS/PDC patients with cognitive dysfunction. Personality change and aphasia were not present, but hallucination (5/12) and abulia (9/12) were recognized in Kii ALS/PDC with cognitive dysfunction.

Conclusions: The neuropsychological features of patients with Kii ALS/PDC were characterized by marked abulia and bradyphrenia, hallucination, disorientation and deterioration of recent memory. Kii ALS/PDC showed features characteristic of frontal-subcortical dementia.

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THEME 4 IMPROVING DIAGNOSIS, PROGNOSIS AND DISEASE PROGRESSION

P43 ALS MORTALITY IN BELGRADE /SERBIA CONTINUES TO RISE: EXAMINATION OF MORTALITY RATES 1992-2009

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Keywords: mortality, trends, epidemiology

Background: A number of studies in a wide range of countries have pointed to a worldwide upward trend in ALS incidence and mortality in last 50 years. In our first epidemiological study of ALS in Belgrade the adjusted mortality rate was low: 0.29 per 100,000 population in 7-year period (1985-1991).

Objectives: The aim of this study was to determine the mortality of ALS patients from the district of Belgrade (Serbia), over a period of 18 years (1992 to 2009), paying particular attention to variation in trends by age and sex.

Methods: Annual age and gender specific mortality rates were calculated using data from hospital in- and out-patient ALS registers at the Clinic of Neurology of Belgrade, and departments of neurology in three additional clinical centers in Belgrade, 1992 to 2009 inclusive. The diagnosis of probable or definite ALS was based on the El-Escorial revised criteria. Ten age groups were used: 0-39; five-year age groups through 40-79; and over 80 years. The mortality rates were calculated by standard procedures. The probability of survival was calculated by Kaplan-Meier method.

Results: During the investigated period, 321 ALS patients were diagnosed in the district of Belgrade, Serbia. There were 192 (59.81%) males and 129 (40.19%) females, sex ratio of 1.49:1. The mean age at the onset of ALS was 57.74 ± 11.46 years (range 18-83), for males 56.97 ± 12.11 and for females 58.89 ± 10.36, without statistically significant difference between males and females. Altogether, 267(83.2%), 160 (59.9%) males and 107 (41.1%) females, died before December 31 2009. The overall mortality rate (/ 100,000) was 0.92, 1.16 for males, and 0.71 for females, with statistically significant difference between males and females ($p < 0.01$). The average mortality rate (/ 100,000) of ALS during the same period changed in both groups from 0.19 (1992) to 1.58 (2002), with the peak of 2.80 in 2002 for males, and 1.82 in 2001 for females. During the observed period the mortality rate of ALS in Belgrade showed increasing tendency ($y = 0.524 + 0.023x$, $p = 0.087$). When all ages were analyzed for every year, age specific mortality rates were generally low under the age of 50, rising to a peak of 65-69 years and falling for the over 80 years age group. The median survival duration was 4.145 years, 4.223 for males and 4.011 years for females, without significant difference between them.

Discussion and conclusion: Results showed that ALS mortality increased in Belgrade (Serbia) from 1992 to 2009, with the peak at 2001, for females and at 2002, for males. The most important contributing factors are better diagnosis and registration of ALS, as well as increase of life expectancy. However, real increase in the mortality of our ALS patients related to environmental factors, cannot be excluded.

P44 THE DIAGNOSTIC PATHWAY AND INTERVAL OF AMYOTROPHIC LATERAL SCLEROSIS IN JAPAN

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Background: There have been several reports from different countries concerning the time to diagnosis in patients with amyotrophic lateral sclerosis (ALS). However, little is known about the relationship between the diagnostic interval and pathway in ALS.

Objectives: We sought a correlation between the diagnostic pathway and interval in ALS patients.

Methods: We made a retrospective hospital-based study, and compared the diagnostic pathway and interval in patients diagnosed as having ALS between January 1990 and April 2011. Patients were diagnosed with definite or probable ALS during this period according to the El Escorial criteria. They were stratified according to onset symptoms of ALS (bulbar (BO) and limb onset (LO)). We investigated the speciality of the first consulting physician. The time lapse was calculated between the first symptoms to the final diagnosis of ALS. Statistical analysis was carried out with the Mann-Whitney U-test.

Results: A total of 190 patients fulfilled the El Escorial criteria of ALS. The age of onset varied from 26-83 years and the average age (SD) was 60.1 (11.1). The onset form was 76 (40%) in BO and 114 (60%) in LO. The first physician visit (BO: LO) was 45%: 23% to a neurologist, 17%: 35% to a general practitioner, 13%: 35% to an orthopedist, 10%: 1% to an otolaryngologist and 10%: 5% to a neurosurgeon. The diagnostic interval (SD) from the initial symptoms was 9.0 (5.1) months in BO patients and 15.9 (5.3) in LO patients. The diagnostic interval (SD) by different pathways (BO and LO) was 7.1 (3.2) and 11.3 (4.8) months via a neurologist, 7.0 (5.3) and 13.8 (4.1) months via a general practitioner, 11.3 (4.3) and 20.2 (7.4) in an orthopedist, 8.3 (3.3) and 13.9 (4.4) months in an otolaryngologist and 9.1 (4.5) and 13.3 (3.3) months via a neurosurgeon. There were no statistical differences of diagnostic intervals by the first visit patterns among BO patients. With respect to LO patients, the diagnostic

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interval of an orthopedic visit was longer than that of LO patients visiting a neurologist.

Discussion: The diagnosis of ALS is achieved by examination and a series of investigations designed to exclude other clinical syndromes. One of the most important things in ALS patients is an early diagnosis so that the rapid diagnosis provides the best quality of life for ALS patients and their caregivers. The present study indicated that more than half of ALS patients did not visit a neurologist at the first consultation. As compared to LO patients visiting a neurologist, the final diagnosis of ALS was delayed for more than 6 months in LO patients visiting an orthopedist.

Conclusion: LO patients visiting an orthopedist as the first physician took a longer time to be diagnosed than LO patients visiting a neurologist.

P45 DOES RETROVIRUS INFECTION CAUSE AMYOTROPHIC LATERAL SCLEROSIS?

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Background: The cause of sporadic ALS is unknown. Retroviral infection as a cause of ALS has been proposed because of the HTLV-1 associated corticospinal tract disease and presence of reverse transcriptase activity in sera of ALS patients. Over the last 20 years, 25 cases of ALS or ALS-like syndrome have been reported in HIV-1 (HIV) infected individuals; however, the causal relationship of HIV infection to ALS is uncertain.

Objective: To describe 5 cases of ALS in patients with HIV infection and review the previously reported similar cases in order to compare and contrast with classical sporadic ALS.

Setting: A multidisciplinary ALS center and Neuro-AIDS clinic at a tertiary care university hospital.

Methods: We investigated and prospectively monitored five patients who had developed ALS during the course of their HIV disease. These five cases and 20 previously reported cases were categorized by El Escorial criteria for the level of certainty of ALS diagnosis. The clinical features, disease course, laboratory findings, and response to therapy in HIV-associated ALS were reviewed for comparison and contrast with the characteristics of sporadic ALS.

Results: The clinical course of ALS in our five patients resembled clinically definite ALS in two cases, primary lateral sclerosis (PLS) in two cases, and progressive muscular atrophy (PMA) in one case. Some of these cases are reported elsewhere (J Neurol Sci 2006;240:59–64; Neurology 2008;70:575–77). There was clinical or laboratory evidence of neural structure involvement outside motor system in two cases. Two patients (one each of PLS and PMA) improved in motor deficit following effective retroviral therapy. A review of 20 other reported cases revealed clinically definite ALS in 6 cases and probable or possible ALS in 14 cases. Motor deficit commenced at different stages of the HIV disease; in 7 cases, HIV infection was discovered at the time of ALS diagnosis. CD4 cell count ranged from 2 to 560 cells/mm^3. HIV-associated ALS syndrome generally occurred at younger age, clinical progression was faster, and 8 of 10 cases responded to antiretroviral therapy. Autopsy findings in three fatal cases (one definite ALS) exhibited pathology outside the motor neuron pool.

Conclusions: ALS in patients with HIV infection can be a fortuitous occurrence or a retrovirus-driven ALS-mimic syndrome. Five cases of HIV infection in our cohort of approximately 1450 ALS cases over a 12-years period is comparable to the background HIV prevalence in South Florida. The El Escorial diagnosis in the majority of HIV-associated ALS did not rise to clinically definite level of ALS. Although a causal relationship of HIV infection to classical ALS is uncertain, the recovery following effective antiretroviral therapy in many patients underscores the importance of recognition and treatment of ALS-mimic syndrome in HIV-seropositive individuals.

P46 DO MOTOR NERVE BIOPSIES HELP SEPARATE ALS/MND FROM MOTOR NEUROPATHY?

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Background: The diagnosis of multifocal motor neuropathy with conduction block is defined by the presence of conduction block in two or more nerves in areas not prone to compression. Identifying patients with MMN without conduction block lacks such a specific signature yet IVIG responsiveness is just as robust.

Objective: Describe the spectrum of pathological changes in "motor nerves" in patients with MMN/CB and IVIG responsive MMN without CB. We also identified patients with amyotrophic lateral sclerosis (ALS) to describe findings on motor nerve biopsy in these patients.

Methods: A retrospective study was performed of all patients having a biopsy of motor nerve (median supplying the pronator or obturator supplying the gracilis) from 2001 and 2008.

Results: Fifty two patients were identified. Seventeen had ALS; 11 had MMN with multifocal conduction block. Others had motor neuropathy without definite multifocal conduction block ($n = 4$) and other neuromuscular disorders ($n = 21$). Thin large diameter axons were prominent in 4/11 MMN patients and 1/17 ALS patients (greater than 30% of all large myelinated axons were thinly myelinated). Large groups of small axons (groups = 5 axons or more clustered together; small axons: < 4.5 microns in greatest diameter) occurred in 7/11 MMN patients with a predominance of groups being composed of small axons. No patient had large clusters of mixed large and small axons composed of greater than 8 axons of nerve fibers and thinly myelinated axons were rare compared to fascicles of ALS. Onion bulbs were seen in 2/11 patients. Thinly myelinated fibers and large groups of axons may support the presence of MMN and not ALS.

All patients with motor neuropathy had prominent findings in one or more of the following: Large groups of small diameter fibers, focal loss of large diameter axons, and axonal clusters. No patient with ALS had multiple findings and only one had moderate amounts of groups. Our findings suggest

that motor nerve biopsy showing large axonal clusters of greater than 8 axons composed of a mixture of numerous large thinly myelinated axons and associated small axons strongly supports the presence of MMN and not ALS. Small axonal clusters or patchy large groups of small axons is more supportive of ALS.

P47 DEFINING FAMILIAL ALS: A WORLDWIDE SURVEY

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Introduction: Familial amyotrophic lateral sclerosis (FALS) is clinically indistinguishable from sporadic amyotrophic lateral sclerosis (ALS). A recent meta-analysis reported that the rate of familial amyotrophic lateral sclerosis (FALS) among ALS cases was 5%. Only 6% (2/33) of studies included in the meta-analysis provided a definition for FALS.

We hypothesized that the definition for FALS differs among neurologists working in the field of ALS.

Method: We set out to test this hypothesis by designing and administering an online questionnaire to clinicians involved in the diagnosis and management of ALS.

Results: There were 78 respondents from 15 countries. 62.3% of respondents were male. 71.6% of the respondents were neurologists with the remainder being traineeneurologists and clinical geneticists. 84.5% had a special interest in ALS.

Respondents were asked whether or not they thought that there was a standard definition among neurologists for FALS. One-third of total respondents thought that neurologists were using the same definition for FALS (32.4%, 23/71). There was a statistically significant difference when subgroup-analysis based on country of practice was carried out: 52%(13/25) of respondents from North America thought that there was a standard definition for FALS in use among neurologists in comparison to 19.4% (7/36) of respondents from Europe ($p = 0.017$).

Respondents were given five definitions for FALS and asked which matched the definition for FALS that they use in the clinical setting. There was no consensus among the respondents but the preferred definition for FALS was 'a patient with ALS with either a first or second degree relative also with ALS (39.7% 27/68).

Respondents were then asked to look at eight pedigrees and give their clinical opinion on whether the kindred constituted FALS or not.

In cases where there was a clear autosomal dominant pattern of inheritance the level of consensus was high. In over half of the pedigrees more than a third of respondents chose the maybe option. 60.6% (43/71) respondents gave answers that differed to the definition that they had previously stated that they used in clinical practice.

65.3% (47/72) of respondents stated that they routinely carry out genetic testing on patients who had a family history of ALS.

80.3% (57/71) of respondents agreed that a consensus meeting would be helpful in defining FALS.

Conclusion: The results of this study show that there is no clear consensus among neurologists as to what constitutes FALS. Respondents agree that there is a clear need for a consensus meeting on the standard definition of FALS. A standard definition for FALS will make entry into epidemiological and genetic studies more transparent and will in turn permit more accurate comparison between geographic regions.

P48 A MODEL FOR PREDICTING ALS DISEASE PROGRESSION

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Keywords: prognosis, phenotypes, ALSFRS-R

Background: Studying the frequencies that patients with phenotypes marked by isolated limb involvement progress into phenotypes that also affect respiratory and bulbar muscles could be useful in developing predictive outcome models for ALS. The transition from limb-only to bulbar or respiratory phenotypes can then be used to model survival, directly from the time that the respiratory or bulbar decline begins.

Objectives: To present the first part of this predictive model. The model, based on the ALSFRS-R, first defines "limb-only" impairment states, and then uses them to predict transit times to "late" respiratory and bulbar phenotypes.

Methods: We used data from 412 subjects in the WALS clinical trial of minocycline. We defined a limb-only phenotype when ALSFRS-R scores suggested the total absence of respiratory or bulbar impairment (at enrollment). This included 125 patients who had respiratory and bulbar subscale scores of 11 or 12 and FVC > 90% at entry.

This limb-only group was then further subdivided into four subgroups based on the gross and fine motor subscores. The groups were defined as follows: L0: no impairments (i.e. FINE and GROSS = 11 or 12), L1: fine impairment alone (i.e. FINE < 11, GROSS = 11 or 12), L2: gross impairment alone (FINE = 11 or 12, GROSS < 11), and L3: impairment in both. We then determined the transit times to the onset of respiratory or bulbar decline (defined as these subscores falling to 10 or less or by the FVC dropping by 10% or more from its baseline.

Results: The 125 patients included 4 L0; 21 L1; 17 L2; and 83 L3 (for further analysis, we did not include L0 since the number was too small). Those in L3 proved to be the fastest decliners in each of the three categories. At six months, FVC drops of at least 10% from baseline had developed in (L1, L2, L3) 63%, 48%, 28%; bulbar subscore declines in 44%, 43%, and 17%; and respiratory subscore drops in 48%, 43%, and 11%. Statistically, testing over the whole study duration of 13 months, times to FVC drop did not differ significantly ($p = 0.17$) by ANOVA of the three groups, while those for respiratory ($p = 0.01$) and bulbar ($p = 0.02$) were significant. Interestingly, on direct comparison of FINE V GROSS, L1 had greater risk for respiratory ($p = 0.08$) and bulbar ($p = 0.09$) impairment than L2.

Discussion: Isolated gross motor impairment predicts the longest transit time to respiratory and bulbar involvement, likely reflecting the distance of lumbosacral myotomes from respiratory or bulbar muscles. More importantly, our work suggests that clinical phenotypes can be determined from the FRS can be useful for predicting outcomes. This work is only one step in a larger model that considers the survival once respiratory or bulbar begin to decline and accounts for the rate of progression and other baseline findings to predict survival.

P49 PREDICTORS OF INCREASE IN SEVERITY AMONG JAPANESE AMYOTROPHIC LATERAL SCLEROSIS PATIENTS BY DISCRIMINANT ANALYSIS

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Keywords: discriminant analysis, prognosis, prevention

Objective: It is important to select individuals at high risk of increase in severity of amyotrophic lateral sclerosis (ALS) earlier to maintain quality of life and mental health for both patients and their caregivers. Prognostic factors for increase in severity have not been well documented. We constructed a predictive model to discriminate patients at a higher risk of increase in severity in Japanese ALS patients, using linear discriminant analysis.

Methods: Data were collected for 183 ALS patients diagnosed by EI Escorial World Federation of Neurology criteria who got informed consent to participate in a case-control study and the follow-up one year later. A structuredself-administered questionnaire specifically designed for this case-control study was distributed and collected by mail in both patients and controls. We asked patients to recall their lifestyle within the 3 years before the onset of ALS. Assessment of increase in severity was compared between joining the study and one year later based on self-report of caregivers. Change in increase in severity was categorized into two groups: stable (better or same) and worse. Stepwise linear discrimination analysis was used to construct a predictive model to select individuals who have a higher risk of increase in severity, using variables with a significant difference of $p < 0.05$ by t-test and chi-square test.

Results: Proportion of males, age at onset (age 65 and over), short duration of ALS, much mental imbalance, type A behaviour, symptom at ALS onset, i.e. bulbar onset and limb onset, much stress, less daily intake of green-yellow vegetables and loss of purpose in life were significantly higher in worse group than in stable group. Among those factors, the stepwise discriminant analysis selected the 3 predictor variables (male, short duration of onset, much mental imbalance) and yielded a statistically significant function ($\lambda = 0.5$; $\chi^2 = 10.5$, df.3, $p < 0.001$). This function showed that the rate of correct prediction was 89.6 % for change in increase in severity.

Conclusion: The calculated discriminate function based on the above 3 predictor variables (male, short duration of onset, much mental imbalance) is useful for detecting individuals at high risk of increase in severity and preventing its development among ALS patients. Prospective studies are needed to confirm the validity and feasibility of the model for earlier screening for increase in severity among ALS patients.

P50 GLUCOSE AND HIGH DENSITY LIPOPROTEIN: POOR PROGNOSTIC FACTOR IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: glucose, high density lipoprotein, prognosis

Background: Studying the energy metabolism of amyotrophic lateral sclerosis (ALS) patients leads to better understanding of the pathogenesis of ALS and offers a new perspective to the treatment for ALS patients. Plasma markers of energy metabolism were identified in ALS patients in Southwest China.

Methods: A total of 138 ALS patients (85 males and 53 females), from the Department of Neurology of West China Hospital of Sichuan University from 2005-2010 were included in the study. Venous blood samples were collected after 12-hour overnight fasting and tested in the lab of West China Hospital of Sichuan University. Information on functional rating scale scores was provided by family members, caregivers, and family physicians.

Results: The mean onset age of the patients was 50.35 ± 12.36 years and the mean disease duration was 19.35 ± 17.23 months. Ninety-one patients were spinal onset and 47 patients were bulbar onset. Overall, the mean levels of uric acid (304.25 ± 68.10 μmol/L), triglyceride (1.61 ± 0.53 mmol/L), cholesterol (4.85 ± 0.90 mmol/L mmol/L), high density lipoprotein (1.42 ± 0.26 mmol/L), low density lipoprotein (2.84 ± 0.62 mmol/L), and glucose (4.79 ± 0.57 mmol/L) were normal in plasma. However, the high density lipoprotein ($P = 0.001$, $R = 0.58$) and glucose ($P = 0.002$, $R = 0.69$) were correlated with onset age and the glucose was negatively correlated with functional rating scale score ($P = 0.006$, $R = -0.62$).

Conclusion: Low level of glucose and high density lipoprotein may be an important risk factor for decreased survival time.

P51 CSF NEUROFILAMENT LIGHT CHAIN LEVELS AT DIAGNOSIS CAN PREDICT PROGRESSION TO GENERALIZED AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: biomarkers, generalization time, prognosis

Background: The natural history of ALS is characterized by clinical heterogeneity. Biomarkers are needed to improve prediction of prognosis. Neurofilament subunits (NFs) are the major structural components of axons and CSF neurofilament light chain (NF-L) levels have been proposed to monitor motor neuron damage. In ALS the prediction of prognosis is usually based on death as the outcome measure.

The conversion from bulbar or spinal ALS to generalized ALS is a critical moment and an alternative prognostic outcome.

Objective: To evaluate if CSF NF-L levels may predict the time to conversion to generalized ALS.

Methods: NF-L assay was performed in CSF samples. A two-site solid phase sandwich ELISA was used to measure NF-L levels. This method has a detection limit of 31 ng/L with a variability intra assay CV% < 6 and inter assay CV% < 9. Neurological status was assessed by revised ALS Functional Rating Scale (ALSFRS-r). We considered the "time to conversion to generalized ALS (TTG)" as the time of symptoms spreading from spinal or bulbar localization to both. We used the median CSF NF-L to dichotomize the sample (subjects with CSF NF-L levels above or below the median). Spearman test was used to assess correlations between variables. Kaplan-Meier analysis (Log-rank method) was used to compare the effect of CSF NF-L levels on TTG. Cox regression model was used for univariate and multivariate analysis (adjusting for age, gender and ALSFRS-r at baseline).

Results: We enrolled 37 sporadic ALS patients with a median age of 55.5 years (range: 36.5-76.8), median disease duration at time of lumbar puncture of 12.1 months (range: 2.2-142.5). The median of NF-L levels was 5000 ng/L (range: 668.7-10000). The median of the TTG was 16.5 months (range: 0-79). CSF NF-L levels were inversely correlated to TTG ($rs = -0.65$; $p = 0.0001$). Kaplan-Meier analysis showed shorter TTG in patients with high NF-L levels (Log-rank test Chi-squared = 14.8, $p = 0.0001$). In the Cox regression model NF-L levels predict the TTG with a HR = 1.28 (95% CI 1.11-1.48, $p = 0.0006$). Multivariate Cox regression model suggested that patients with high NF-L levels have a risk five times higher than patients with low NF-L levels (HR = 5.8, 95% CI 2.2-16.7, $p = 0.0005$) to progress to generalized ALS.

Discussion and conclusion: In this pilot study CSF NF-L levels predict a shorter time to conversion from symptom onset to generalized ALS. The use of TTG as an intermediate end point could give us information about the progression in the early stages of the disease. Further studies are needed to confirm these preliminary results using CSF-NF-L as a biomarker of disease progression.

P52 THE IMPAIRMENT OF THE OXIDATIVE STATUS CORRELATES WITH SEVERITY OF THE DISEASE IN ALS PATIENTS

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Keywords: oxidative status, thiols, severity of disease

Background: Several mechanisms have been proposed to account for the progressive motor neuron degeneration, including oxidative stress, neurofilament damage, mitochondrial abnormalities, glutamate-mediated excitotoxicity, and altered responses to hypoxia. Since increased levels of oxidative stress have been found not only in nervous but also in peripheral tissues of familial and sporadic ALS patients, many studies have been addressed to relate the presence of increased oxidative stress to reduced antioxidant defenses also in peripheral tissues of these patients. However, the majority of these data showed conflicting results, due to patients' heterogeneity in terms of disease time onset and course.

Objective: In this work we assayed the oxidative status in ALS patients. In fact although it is not yet established if oxidative stress is a cause or a consequence of the neurodegenerative process, the different capacity of each subject to respond to increased oxidative stress may account for the heterogeneity of the ALS patients in terms of clinical course, disease duration and response to pharmacological treatment.

Patients and methods: We performed the derivatives-reactive oxygen metabolites (d-ROMs) test for the measurement of free radical metabolites, the biological antioxidant potential (BAP) to assess the antioxidant power of the serum, and the thiol antioxidant barrier (SHp) test for the measurement of total thiol group concentration in ALS patients' sera. We also evaluated the plasma glutathione (GSH), which is the most important intracellular buffer for redox status. To perform the above tests we utilized the commercially available d-ROMs, BAP and -SHp tests (Diacron International, Grosseto, Italy), respectively, and the automated clinical chemistry analyser SYNCHRON, CX9 PRO (Beckman Coulter, Brea, CA, USA). Plasma reduced GSH was determined by high-pressure liquid chromatography (HPLC).

Results: We consecutively recruited 91 patients (mean age 64 + /-12 years; median disease duration 35 months) at NEuroMuscular Omnicentre (NEMO) in Milan. In 69% of patients the d-ROMs test showed a marked increment of free radical metabolites. Moreover, in 83% of ALS patients the SHp test showed a severe reduction of total thiol groups concentration. Finally, all of the above markers were not correlated with the clinical features of ALS patients, except for the plasma GSH concentration that was significantly correlated with disability, as measured by ALSFRS-r total score ($r = 0.46$, $p = 0.002$).

Discussion: Our results showed a significant impairment of oxidative activity with a marked increment of free radical metabolites levels and depletion of thiol levels in the majority of ALS patients. Among the clinical features, only the severity of the disease was strongly correlated with plasma glutathione.

Conclusion: Interestingly, our results are in favour of an oxidative imbalance in the ALS population, from the early phase of disease.

P53 NATURAL HISTORY OF PURE UPPER MOTOR NEURON DISEASE/DYSFUNCTION (PUMND)

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Keywords: PLS, natural history, EMG

Background: Primary lateral sclerosis (PLS) begins with pure upper motor neuron disease/dysfunction (PUMND) and, by definition, it remains as PUMND throughout its entire disease course. According to the current criteria, the diagnosis of PLS cannot be made until PUMND is sustained for at least 36 to 48 months after symptom onset. Our critical review of all the reported PLS autopsy studies in the past suggests that true PUMND based on strict pathological and EMG criteria may be exceedingly rare. When lower motor neuron dysfunction (LUMND) develops in patients with

PUMND, the term UMN-dominant ALS, is applied. How often we encounter PUMND among all new MNDs and how often PUMND evolves into UMN-dominant ALS is not well-known. Understanding the natural history of PUMND appears to be essential for defining the relationship between amyotrophic lateral sclerosis (ALS) and PLS.

Objective: To determine the proportion of PUMND among all newly seen MND/ALS cases and the early evolution of PUMND cases based on clinical and EMG changes.

Methods: We retrospectively reviewed 622 cases of MNDs newly seen in a 4-year period between 2003 and 2007 and identified 33 patients with PUMND (5.3%). We excluded cases with spastic paraplegia or incomplete medical records. Retrospective analyses were made in 22 cases (3.5%) which satisfied PUMND of undetermined cause based on clinical, extensive laboratory studies and electrophysiological data.

Results: Of the 22 cases, 7 cases (32%) were later classified as UMN-dominant ALS (UMN-D ALS) because of new denervation on EMG (on average 50 months after symptom onset and ranging 25 - 102 months). Out of these UMN-D ALS cases, 2 (29% of UMN-D) developed LMND at 82 and 102 months after symptom onset. Thirteen cases (59%) remained as PUMND evidenced by one or more repeated normal EMGs (average 55 months), whereas 2 cases (9%) were based on clinical findings alone (no repeated EMG). Among these PUMND cases, 5 have not reached the 48 month cut-off period to be called PLS.

Discussion and conclusions: Our study shows PUMND is generally rare among MNDs. Although the majority of the PUMND cases developed LMND within 48 months of symptom onset, nearly 30% of these cases developed LMND after 48 months and up to 102 months, implying that LMND may develop in a good proportion of PUMND cases at some point in its course. This observation indeed makes PLS a rare disease. We still do not know if ALS and UMN-D ALS are biologically different, and if UMN-D ALS and PLS are different. To answer these questions, we need a large, prospective, long-term, and multicenter natural history study of PUMND, which is essential to understanding the relationship between ALS and PLS.

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P54 CLINICAL FEATURES IN AMYOTROPHIC LATERAL SCLEROSIS SUBTYPES: A HOSPITAL-BASED REGISTRY STUDY

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Keywords: subtypes, duration to fasciculation, progression

Background: Amyotrophic lateral sclerosis (ALS) is difficult to diagnose, with a progressive fatal course and unknown aetiology.

Objectives: To explore epidemiology and clinical features of different subtypes of ALS.

Methods: Between January 1st 2000 and August 31st 2010, 63 patients who were diagnosed with ALS at Huashan Hospital were recruited. Data on demographics, risk factors, clinical features, physical and neurological examinations, EMG, laboratory, neuroimaging and ALS Functional Rating Scale (ALSFRS) were recorded. Subtypes were classified according to the onset region of ALS, including bulbar, arms and legs. Fisher's exact test was used to compare features of ALS subtypes and multiple regression models were used based on demographic, vascular risk factors, and clinical variables.

Results: We identified 57 individuals (36 male and 21 female, sex ratio = 1.7) from our hospital in-patients with mean age of 51.3 years old. Among them, 19 patients had a history of trauma or surgery, and 42 developed symptoms symmetrically. The mean duration from onset to diagnosis was 18 months. Patients with bulbar-onset were diagnosed earlier, at a mean time of 10.58 months. Patients with arms-onset were younger and fasciculation happened earlier, with higher levels of CPK compared to the other two groups. All groups of patients were similar in ALSFRS-R, suggesting patients with legs-onset developed more slowly. In multi-regression analysis, age and fasciculation were related to ALS subtypes.

Discussion: The results demonstrated the characteristic clinical profiles of subtypes of ALS patients. A striking observation in the present study is that region of onset greatly relates to clinical features, including the age, time to diagnosis, and time to fasciculation.

Bulbar onset accounted for one-fifth of all patients, who were characterized as older aged, male and more likely to be blue-collar. The incidence of bulbar onset in 20% of ALS patients has been reported with onset at an older age and with a poor prognosis in some previous studies. In our observation, they are diagnosed at an early phase, which also revealed fast progression. Patients with limb-onset took a longer time to be diagnosed than those with bulbar-onset. Those with arm-onset are younger than patients of other groups. Patients with arm-onset presented fasciculation earlier, with higher levels of CPK and LDH. In diagnosis, patients with bulbar-onset can be diagnosed earlier than that of limb onset. ALSFRS-R evaluates the daily function of patients, which is closely related to severity of the disease. Patients of each onset region are similar in ALSFRS-R scores, which suggests leg onset patients have the same ALSFRS-R scores as bulbar-onset patients at an early time from diagnosis. It was confirmed ALSFRS-R scores have a strong relationship with prognosis, suggesting bulbar onset with a poor prognosis.

Conclusion: Age of onset and duration to fasciculation were related to ALS subtypes in this cohort.

P55 VALIDATION OF A NEW ALS CLINICAL EVALUATION TOOL, THE MADRID QUANTITATIVE NEUROMUSCULAR ASSESSMENT, MAQUINA

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Keywords: clinical assessment, motor function, deficit quantitation

Background: MAQUINA was designed and proved consistent and reliable to quantify motor deficits in ALS patients (1,2). It is composed of 2 timed test of bulbar (pataka, count), 5 of arm (marbels R + L, pedalling, tapping R + L), and 4 of leg function (3m + 3m walk, pedalling, tapping R + L).

Objective: To analyze the validity of MAQUINA to quantify motor deficit in ALS patients throughout the disease by

comparison with current assessment tools (ALSFRS-R, MMT, MVIC, and FVC/slowVC).

Methods: Sixty-three patients with ALS were assessed by 2 physiotherapists following the standardized MAQUINA protocol every 3 months for one year. At the same times they were evaluated by the ALSFRS-R, MMT, QMA (maximum voluntary isometric contraction of 6 UE and 4 LE selected muscle groups), and FVC/slowVC. Cronbach's alpha was used for internal consistency. Spearman's Rho Index was used to assess test retest reliability. The same Index was used to assess validity by comparing MAQUINA with the other methods. Analyses were done by SPSS15.0.

Results: The results showed high internal consistency (rxy:0.79-0.97) and a good test-retest correlation (0.97, $p < 0.01$). A high correlation between total MAQUINA and ALSFRS-R scores was found at initial assessment (-0.69, $p < 0.01$) and higher at 12 months (-0.80, $p < 0.001$). Other correlations were: the corresponding MAQUINA and ALSFRS-R bulbar tests (items 1 + 2 + 3) at initial assessment (-0.72, $p < 0.01$) and at 12 months (-0.78, $p < 0.001$); arm + leg tests (items 4 to 9) at initial (-0.80, $p < 0.01$) and at 12 months (-0.90, $p < 0.01$); MMT of arms at onset (-0.62, $p < 0.001$) and at 12 months (-0.78, $p < 0.01$); MMT of legs at onset (-0.53, $p < 0.01$) and at 12 months (-0.62, $p < 0.01$). There was correlation between bulbar/respiratory tests of MAQUINA and FVC or SVC at 12 months (-0.43 a -0,50, $p < 0.01$). Correlation with MVIC is also significant: MAQUINA arm tests with elbow flexion and extension (-0.32 to -0.7, $p < 0.01$) and hand grip (-0.5 to -0.7, $p < 0.01$), and MAQUINA leg tests with knee flexion and extension (-0.4 to 0.6, $p < 0.01$).

Conclusions: The MAQUINA timed battery has proved to be a reliable and valid assessment tool to quantify motor deficits over time in ALS patients, either as a single complete test or as three separate components, bulbar/respiratory, arms and legs. Further comparison analysis regarding assessment at onset and end phase of the disease are ongoing.

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P56 AMYOTROPHIC LATERAL SCLEROSIS DASHBOARD: COGNITIVE, BEHAVIORAL, BULBAR, RESPIRATORY, ARM, LEG DOMAIN-SPECIFIC DISEASE STAGING

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Keywords: disease staging, El Escorial, Awaji

Background: ALS Dashboard is a new tool for analyzing disease severity within a single patient and across different patients defining involvement over 6 domains on a per patient basis.

Methods: ALS patients (199/263) at first clinic visit were categorized as El Escorial Criteria clinically definite(EECD) only (90) or Awaji clinically definite only (AwCD) (109) and staged according to the ALS Dashboard criteria. Comparisons of ≥ stage 3 disease in each domain by EECD/AwCD criteria were conducted by chi-square test with Yates correction and confirmed with Fisher's exact test.

At first clinic visit in EECD/AwCD ALS ≥ stage 3 cognitive disease was similar (8.9%/13.8%; $p = 0.3968$), ≥ stage 3 pseudobulbar affect (11.1%/2.8%; $p = 0.0369$), ≥ stage 3 depression (25.6%/10.0%; $p = 0.0070$), ≥ stage 3 bulbar dysfunction (45.6%/13.8%; $p = 0.0001$), ≥ stage 3 arm dysfunction (35.6%/8.3%; $p = 0.0001$), ≥ stage 3 leg dysfunction (55.6%/33.9%; $p = 0.0036$) were statistically significantly increased and ≥ stage 3 respiratory dysfunction was identical (7.8%/4.6%; $p = 0.5209$).

Results: Disease severity in some domains segregates differently with a higher proportion of ≥ stage 3 disease in EECD ALS. The proportion of ≥ stage 3 disease ranks similarly: leg > arm > bulbar > depression > pseudobulbar affect.

Conclusions: Disease severity in some domains, segregates differently with a higher proportion of ≥ stage 3 disease in EECD ALS. The proportion of ≥ stage 3 disease ranks similarly: leg > arm > bulbar > depression > pseudobulbar affect. ALS Dashboard is a new tool for analyzing disease severity within a single patient and across different patients.

P57 DYNAMIC POSTUROGRAPHY EVOKES SENSORY AND MOTOR DEFICITS LEADING TO DISEQUILIBRIUM AND PREDICTS FALLS IN AMBULATORY ASYMPTOMATIC AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Keywords: fall, balance, sensory integration

Background: Falls are common in ALS. Previous observation in our clinic indicates that ambulatory ALS patients who did not fall on condition 4 of the modified sensory integration and balance had excessive sway or fell in condition 5 (C5) and C6 of the computerized dynamic platform posturography - EquiTest (CDP). No comparisons with healthy controls (HC) were available.

Objectives: To identify problems in equilibrium of ALS patients compared to HC.

Methods: Nineteen ambulatory early diagnosed patients with no symptoms or history of fall or motor problems by Berg Balance Scale, Tinetti, dynamic gait index, get-up-and-go, sit to stand, and 25 foot walk tests, and 15 adults matched for age and gender (HC) performed the sensory organization test (SOT) and motor control test (MCT) using CDP. The SOT involved 6 sensory conditions © with 3 fixed support conditions (FS) and 3 sway-referenced conditions (SR); (C1) vision normal, support normal (C2) vision absent, support normal (C3) vision SR, support normal, (C4), vision normal, support SR, (C5) vision absent, support SR, (C6) vision and support SR. Equilibrium scores (ES) for each C ranging from zero (worst = fall) to 100 (best = no sway) were generated to identify sensory dysfunctions and/or abnormal sensory organization patterns (SOP). In total, 18 ES were obtained, 3 for each condition. The arithmetic mean of the 3 scores was used. MCT was evaluated by the latency of time (in milliseconds (ms) to recover from small and large forward and backward translations (2.8 and 8.0 deg/sec).

Results: Compared to HC, ALS patients exhibit: 1) Lower composite ES in C5 (51 ± 22 vs. 65 ± 11 ($P \leq 0.05$); 2) Higher incident of falls during C4-C6. 7/19 (37%) vs. no falls in HC. 2 patients fell on C4, 5 patients on C5, and 6 patients on C6 ($P \leq 0.03$); 3) lower vestibular SOP score among (0.51 ± 0.2 vs. 0.7 ± 0.11, $P \leq 0.03$); 4) Longer latency (165.4 ± 9.9 vs.149.7 ± 9.5); 5) Fallers during CDP reported their first fall during follow-up ALS Clinic visits with a median time to first fall = 120 days sooner than non-fallers (Kaplan-Meier plot, HR = 1.9973, (95%CI = 0.6576 to 6.0664); $P \leq 0.176$)).

Discussion and conclusions: Altered SOP characterized by difficulty to utilize information from the vestibular system leading to disequilibrium and fall is seen early in ALS. The vestibular influence on postural sway depends on peripheral vestibular organs, branches of the vestibular nerve to the vestibular brainstem nuclei and their connection to the cerebellum. Latency problems may indicate extra vestibular, central nervous system lesions. Efficacy of balance, ocular-vestibular and neuromuscular reeducation early in ALS needs to be explored.

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THEME 5 IMAGING, ELECTROPHYSIOLOGY AND MARKERS OF DISEASE PROGRESSION

P58 PATHOPHYSIOLOGICAL INSIGHTS INTO FACIAL ONSET SENSORIMOTOR NEURONOPATHY SYNDROME: A NOVEL SYNDROME IN NEUROLOGY

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Keywords: FOSMN, cortical excitability, neurodegeneration

Background: Facial onset sensory and motor neuronopathy (FOSMN) syndrome is a slowly progressive neurodegenerative disorder of sensory and motor neurons, heralded by development of sensory deficits in the trigeminal nerve distribution, with spread of symptoms to affect the neck, upper trunk and upper limbs in sequential order. Motor deficits develop later in the course of the disease. Although degeneration of sensory and motor neurons underlie the development of FOSMN syndrome, the pathophysiological mechanisms remain to be determined. Recently, the co-existence of upper and lower motor neuron signs in FOSMN syndrome suggested a link between FOSMN syndrome and amyotrophic lateral sclerosis (ALS).

Objectives: Given that cortical hyperexcitability appears to be intrinsic to the pathophysiology of ALS, the present study aimed to assess the pathophysiological mechanisms underlying FOSMN syndrome, and in particular whether cortical hyperexcitability is a prominent feature in FOSMN syndrome.

Methods: Studies were undertaken on five patients with clinical features of FOSMN syndrome, heralded by onset of sensory deficits in the trigeminal nerve distribution. All patients underwent clinical, laboratory, conventional neurophysiological and neuroradiological assessment to exclude potential mimic disorder of FOSMN syndrome. Cortical excitability studies were undertaken using a threshold tacking transcranial magnetic stimulation (TMS) technique utilising a 90 mm circular coil. Recordings of motor evoked potential (MEP) responses were made over the abductor pollicis brevis. Results were compared to 30 age-matched healthy subjects and 104 ALS patients.

Results: There were three male and two female patients with FOSMN syndrome with mean disease duration of 52.3 ± 10.6 months. Upper motor neuron signs were not evident in any of the FOSMN patients. Conventional neurophysiological studies revealed abnormalities of blink reflexes, prolonged or absent R1 and R2 components, in all FOSMN patients tested. Pathological studies disclosed the presence of axonal degeneration with absence of inflammation or amyloid deposition. Cortical excitability studies revealed that the resting motor threshold was increased in FOSMN patients ($71.8 \pm 4.3\%$) when compared to ALS ($57.2 \pm 0.9\%$, $P < 0.001$) and controls ($61.8 \pm 1.6\%$, $F = 8.7$, $P = 0.05$). In addition, averaged short interval intracortical inhibition was increased in FOSMN patients ($6.8 \pm 1.3\%$) when compared to ALS ($2.6 \pm 0.9\%$, $F = 11.2$, $P < 0.05$) but comparable to healthy controls ($10.5 \pm 1.1\%$, $P = 0.09$). Of relevance, there was a significant difference between groups in the cortical silent period duration (FOSMN 204 ± 12.7 ms; ALS 181.3 ± 4.3 ms; controls 215.3 ± 3.8 ms, $F = 8.5$, $P < 0.001$), and MEP amplitude (FOSMN $26.6 \pm 7.0\%$; ALS $38.1 \pm 2.2\%$; controls $25.2 \pm 2.8\%$, $F = 4.7$, $P < 0.05$).

Discussion and conclusions: The findings in the present study suggest that although FOSMN syndrome is a slowly progressive neurodegenerative disorder of sensory and motor neurons, cortical hyperexcitability does not appear to underlie the development of FOSMN syndrome. In addition, the threshold tracking TMS technique clearly differentiated FOSMN syndrome from ALS, thereby arguing against a potential link between these two disorders.

P59 DISSECTING CENTRAL AND PERIPHERAL MOTOR CONTRIBUTIONS TO THE PATHOPHYSIOLOGY OF SPINAL MUSCULAR ATROPHY

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Keywords: transcranial magnetic stimulation, spinal muscular atrophy

Background: Spinal muscular atrophy (SMA) clinically presents as a pure lower motor neuron disorder with muscle weakness and atrophy. Neurodegeneration of spinal motor neurons in SMA is secondary to reduced levels of the survival motor neuron (SMN) protein, which is also expressed in the brain. Whether cortical dysfunction or alternatively, plasticity occurs in response to spinal motor neuron neurodegeneration remains to be determined.

Objectives: To gain further insights into corticomotoneuronal function and disease pathogenesis in SMA, the present study utilised clinical and functional assessments, combined with threshold tracking transcranial magnetic stimulation (TMS) techniques and peripheral nerve studies to investigate SMA patients.

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Methods: Cortical and peripheral nerve excitability studies were undertaken in 11 SMA patients with homozygous deletions in the SMN1 gene (mean age 21.9 years, range 16-36 years) and 24 age matched controls. A comparison was also made with a disease control group, comprising 81 amyotrophic lateral sclerosis (ALS) patients. Motor-evoked potentials and compound muscle action potentials (CMAPs) were recorded from the right abductor pollicis brevis. Neurophysiological parameters were correlated with clinical measures of disease severity.

Results: Motor evoked potential amplitude was significantly increased in SMA when compared to healthy controls, but similar to ALS (SMA $39.7 \pm 4.0\%$; ALS $38.8 \pm 2.8\%$; controls $20.3 \pm 2.5\%$; $F = 10.1$, $P < 0.0001$). In contrast, short interval intracortical inhibition (SMA $14.4 \pm 1.6\%$; ALS $4.3 \pm 1.8\%$; controls $17.0 \pm 2.3\%$, $F = 11.4$, $P < 0.0001$) and cortical silent period duration (SMA, 204.4 ± 9.8 ms; ALS, 182.7 ± 5.2 ms; controls 208.8 ± 3.7 ms, $F = 4.8$, $P = 0.01$) were similar between SMA patients and healthy controls, but significantly larger when compared to ALS. Of relevance, peripheral disease burden, as measured by the CMAP amplitude (SMA, 6.3 ± 0.8mV; ALS, 5.9 ± 0.4 mV; controls, 11.8 ± 0.5 mV, $F = 35.5$, $P < 0.0005$) and neurophysiological index (SMA, 0.7 ± 0.2; ALS, 0.7 ± 0.1; controls, 3.1 ± 0.2, $F = 108.2$, $P < 0.0005$), were significantly reduced in both SMA and ALS patients when compared to healthy controls.

Discussion and conclusions: Simultaneous assessment of central and peripheral motor pathways has established degeneration of the lower motor neuron axis. Despite this process, there remains preservation of corticomotorneurons in SMA, refuting any significant physiological effects of reduced SMN protein expression within the CNS. These findings suggest a unique pathophysiology for motor neuron degeneration in SMA, particularly when compared to ALS. Further, the descending corticomotorneuron output is preserved in SMA and consequently its greater contribution onto the surviving spinal motor neurons likely represents adaptive neuroplasticity.

P60 UPPER MOTOR NEURON ABNORMALITIES IN PARRY-ROMBERG SYNDROME

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Keywords: Parry Romberg Syndrome, transcranial magnetic stimulation, diffusion tensor imaging

Background: Parry-Romberg Syndrome (PRS) is a clinically heterogenous disorder characterised by hemifacial atrophy, and variable associated intracerebral abnormalities that produce contralateral neurological manifestations, including hemiparesis, hemianopia and focal seizures. The aetiology of PRS is unknown, but there are a few reported associations with scleroderma.

Objectives: A subject with PRS had assessment of the upper motor neurons (UMN) with diffusion tensor imaging (DTI) and transcranial magnetic stimulation (TMS) to identify abnormalities in the motor cortex and in the corticospinal tract. The data from this patient with PRS is also compared with normal subjects and subjects with UMN abnormalities as a part of Amyotrophic Lateral Sclerosis (ALS).

Methods: TMS was applied to the motor cortex by a 90mm circular coil connected to a BiStim device, with magnetic evoked potentials recorded from abductor pollicis brevis. Threshold tracking was performed using a paired-pulse paradigm with a conditioning stimulus delivered at intervals before a suprathreshold test stimulus. MRI studies were performed using a 3 Tesla Philips Intera system (Philips Medical Systems, Best, The Netherlands) with an eight channel, phased array head coil and gradient coils (0-33mT/m).

Results: There were differences between the right and left cerebral cortex in PRS as measured by TMS. The Resting Motor Threshold (RMT) was higher in the right hemisphere (90%) than in the left hemisphere (74%), while the Central Motor Conduction Time (CMCT) was prolonged in the right hemisphere (7.95 ms) than in the left hemisphere (3.7 ms). In addition, the Motor Evoked Potential amplitude in the right hemisphere (1.2 mV) left hemisphere (4.4 mV) and Short-Interval Intracortical Inhibition were lower in the right hemisphere (-5.58% threshold change) left hemisphere (4.25% threshold change).

MRI examination of the brain identified generalised atrophy of the right cerebral hemispheres, while the left cerebral cortex was anatomically normal. In addition, DTI studies disclosed a significant reduction of fractional anisotropy using fiber tracking (left cerebral peduncle = 0.64, right cerebral peduncle = 0.5) in the PR patient and (left cerebral peduncle = 0.64 and right cerebral peduncle = 0.60) in the control group and increase in perpendicular diffusivity in the PR patient (left hemisphere 0.49×10-3 mm^2/s and 0.38×10^{-3} mm^2/s in the right hemisphere) when compared to controls in the right hemisphere (left hemisphere 0.375×10^{-3} mm^2/s and right hemisphere 0.41×10^{-3} mm^2/s).

Discussion: The findings in the present study indicate anatomical and functional abnormalities of the right cerebral hemisphere contralateral to the side of deficit. Interestingly, cortical function was preserved in the left hemisphere, the side ipilateral to the limb deficits. The finding of cortical dysfunction is most consistent with developmental abnormalities, possibly occurring prior to decussation of pyramidal fibers *in utero*. Taken together, these findings seem to suggest that Parry-Romberg syndrome may be a developmental disorder occurring during organogenesis in some patients.

P61 CLINICAL AND NEUROPHYSIOLOGICAL EVIDENCE OF MOTOR NEURON DYSFUNCTION IN FRONTOTEMPORAL DEMENTIA

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Keywords: biomarkers, frontotemporal dementia, transcranial magnetic stimulation

Background: Frontotemporal dementia (FTD) and motor neuron disease (MND) share clinical and pathological characteristics. MND develops in some patients with FTD, but the incidence, severity and functional significance of motor dysfunction in FTD has not been determined.

Objectives: To identify and characterise clinical and neurophysiological evidence of motor neuron dysfunction in FTD.

Methods: We performed detailed clinical and neurophysiological assessments on 40 consecutive FTD patients, 42 age/gender matched MND patients and 26 control subjects. The neurophysiological index (NI) was used to detect lower motor neuron dysfunction. Short interval intracortical inhibition (SICI), measured using paired pulse threshold tracking transcranial magnetic stimulation, was used to document upper motor neuron dysfunction.

Results: Of 40 FTD patients, 5 (12.5%) developed concomitant MND (FTD-MND) during the course of the study. A further 9 (27.3%) FTD patients showed clinical evidence of motor neuron dysfunction (ie wasting, fasciculations or weakness), which was generally mild. The neurophysiological index was reduced in FTD (1.1 +/-0.9) compared to controls (1.9 +/- 0.8, $P < 0.001$), but relatively preserved compared to MND (0.7 +/- 0.6, $P < 0.05$). Average SICI was reduced in FTD (4.3 +/-1.7%) compared to controls (9.1 +/-1.1%, $P < 0.05$), and similar to MND: it was particularly reduced in patients with FTD-MND and progressive non-fluent aphasia, but was normal in other FTD variants.

Conclusions: In this cohort of 40 consecutive FTD patients, 5 (12.5%) developed FTD-MND and a further 9 (27.3%) had clinical evidence of mild motor neuron dysfunction. Neurophysiological evidence of lower and upper motor neuron dysfunction was common, even in FTD patients with no clinical evidence of MND. Upper motor neuron dysfunction was most marked in patients with progressive non-fluent aphasia.

P62 THE SPLIT HAND INDEX: A POTENTIAL DIAGNOSTIC TEST FOR ALS

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Keywords: novel, diagnostic, tool

Objective: Preferential atrophy of the Abductor Pollicis Brevis (APB) and the First Dorsal Interosseous (FDI) is a classical feature of amyotrophic lateral sclerosis (ALS), and termed the *split-hand*. The present study assessed the diagnostic utility of a novel neurophysiological measure, the *split-hand index (SI)*, in ALS and in particular, whether SI could reliably differentiate ALS from mimic disorders.

Methods: 20 ALS and110 non-ALS patients were recruited into the study according to the Standards for Reporting of Diagnostic Accuracy (STARD) criteria. Baseline-peak compound muscle action potential (CMAP) amplitudes were recorded over the APB, FDI and Abductor Digit Minimi (ADM) muscles. The SI was calculated as follows:

$$SI = (CMAP_{APB\ AMPLITUDE} \times CMAP_{FDI\ AMPLITUDE}) / CMAP_{ADM\ AMPLITUDE}$$

Results: SI was significantly reduced in ALS patients compared to controls (ALS 3.2 ± 0.6; non-ALS 9.0 ± 0.5, $P < 0.0001$). Analysis of receiver operating characteristic curves suggested that SI reliably and robustly distinguished ALS from mimics (area under curve 0.90, $P < 0.0001$). The optimal SI value for differentiating ALS from non-ALS was 5.2, with a sensitivity of 82%, specificity of 81%, positive likelihood ratio 4.4 and negative likelihood ratio of 0.23. Of relevance, the SI correlated with established clinical (APB Medical Research Council score, $R = 0.7$) and neurophysiological biomarkers ($R = 0.44$).

Conclusion: Findings from the present study establish that the split hand index is a simple and robust diagnostic neurophysiological test clearly differentiating ALS from mimic disorders and may enable earlier diagnosis in ALS and therefore institution of neuroprotective therapies and recruitment into clinical trials.

P63 THE "SPLIT-HAND" PATTERN OF ALS: AN ELECTRODIAGNOSTIC SIGN OF ALS?

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Keywords: nerve conduction study, intrinsic hand muscles, ulnar neuropathy

Background: The "split-hand" pattern (SHP) is an electrodiagnostic (EDx) finding of predominant denervation in thenar and/or first dorsal interosseus (FDI) muscles relative to hypothenar muscles, with normal sensory responses. Although reported as highly suggestive of ALS/MND, an axon loss ulnar neuropathy causing more denervation in FDI than in hypothenar muscles could result in SHP.

Objective: Determine frequency of the SHP in ALS and in ulnar neuropathies.

Methods: Median and ulnar nerve EDx studies in Cleveland Clinic EMG laboratory during five-year period of ALS patients (definite or probable by El Escorial criteria) and of patients with ulnar neuropathy were reviewed. Compound muscle action potentials (CMAPs) were recorded from thenar muscles (stimulating median nerve at wrist), and from FDI and hypothenar muscles (stimulating ulnar nerve at wrist). Sensory nerve action potentials (SNAPs) were recorded from 2nd and 5th digits antidromically, stimulating median or ulnar nerve at wrist, respectively.

Results: 154 EDx examinations recording CMAPs from 252 hands of 142 ALS patients revealed: 1) low CMAPs in FDI, hypothenar and thenar muscles in 32% (81/252), 2) low thenar and FDI CMAPs with normal hypothenar CMAPs in 14% (35/252), 3) low FDI CMAPs alone in 9.5% (24/252), 4) low thenar CMAPs alone in 5.5% (14/252), 5) low hypothenar CMAPs alone in 0% (0/252), 6) normal CMAPs in all three muscle groups in 26% (66/252). Of 81 hands with diffusely low CMAPs but hypothenar CMAPs $\geq$ 2mV, the FDI CMAP was $<$ 50% of hypothenar CMAP in 17 hands (21%). Ninety ulnar neuropathies (in 82 patients) revealed that CMAPs were normal in 17% (15/90), low in FDI in 11% (10/90), in ADM in 13%, and in both FDI and ADM in 59%. Ulnar SNAPs were normal in 24% (22/90); of these, CMAPs were normal in 27%, low in FDI in 18% (4/22), in ADM in 14%, and in both FDI and ADM in 36%. Of cases with only low FDI CMAP, lesion localization was at/distal to the wrist in 33% (3/10), at the forearm in 20%, at/proximal to the elbow in 20%, and not possible in 33%. Three of 4 cases with low FDI CMAPs alone and normal SNAPs were due to lesions in the hand.

Discussion: In ALS/MND patients, CMAPs were preferentially low in lateral compared to medial hand muscles in 36% of hands (90/252). In contrast, only 4% (4/90) of ulnar neuropathies met SHP definition with preserved ulnar SNAPs; all but one was from a lesion in the hand.

Conclusions: Approximately 1/3 of patients with definite or probable ALS have the SHP. It can occur in ulnar neuropathies but is only a "partial" SHP with sparing of thenar muscles. However, limited EDx abnormalities would differentiate these cases from ALS and further support the SHP as highly suggestive of ALS.

P64 THE DISTRIBUTION OF ELECTRODIAGNOSTIC ABNORMALITIES IN EARLY AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: diagnosis, electromyography, neurophysiology

Introduction: Since the Awaji-shima consensus criteria, detection of neurogenic change on needle electromyography has been added to the clinical El Escorial diagnostic criteria for amyotrophic lateral sclerosis (ALS) as evidence of lower motor neuron involvement. In patients with clinically suspected ALS, the relative weighting of upper and lower motor neuron involvement can make diagnosis difficult. Hence it is important that electromyography is focussed on the muscles of highest yield in order to maximise the sensitivity of detection of neurogenic change in segments where lower motor neuron involvement is not readily apparent clinically.

Methods: Electrodiagnostic and clinical data were collected prospectively from 142 patients with clinically suspected ALS in a specialised neurophysiology department attached to a tertiary referral ALS clinic. The involved segments at onset and relative distribution of clinical changes at the time of electrodiagnostic testing were recorded. A standard series of sensory and motor nerve conduction studies and electromyography sampling was performed.

Results: Electrophysiological data was obtained a mean of 14 months after symptom onset. Tibial nerve motor responses were abnormal in 25% of nerves (33% of patients) with reduced compound muscle action potential (CMAP) amplitudes. In the upper limbs, median motor nerve response from APB was abnormal in 71% of nerves tested (74% of patients), Ulnar motor response to abductor digiti mini was abnormal in 38% and to first dorsal interosseous in 71% of nerves. Terminal motor latency was significantly reduced in nerves with reduced CMAP amplitude compared with those of normal amplitude in each of the motor nerves. Tibial, median and ulnar nerves with reduced CMAP amplitude demonstrated significantly reduced persistence of F-waves when compared with normal nerves, while minimum F-wave latencies were significantly prolonged in tibial and median nerves with reduced CMAP amplitudes. Sensory nerve conduction values were abnormal in 11% of patients, most commonly with findings consistent with mild axonal sensory neuropathy. A total of 672 muscles were sampled using standard electromyographic techniques. Combined active and chronic denervation were detected in 10.6-65.4% of sampled muscles, with highest rates in APB, first dorsal interosseous and tibialis anterior muscles. Rates of detection of isolated chronic denervation ranged from 62.7-80.4%. The patterns of nerve conduction and electromyographic abnormalities were analysed in relation to the region of onset, and variation in the pattern of abnormalities was seen.

Discussion: The patterns of electromyographic abnormality described in this study may assist in planning an electrodiagnostic study based on the region of clinical onset in order to maximise the rate of detection of neurogenic change. Additionally, selective large motor neuron loss was suggested by prolongation of distal motor and minimum F-wave latencies in nerves with neurophysiological evidence of axonal loss.

P65 PROGRESSIVE AXONAL DYSFUNCTION UNDERLIES CLINICAL IMPAIRMENT IN ALS

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Keywords: clinical trials, axonal excitability, biomarker

Axonal excitability studies undertaken in amyotrophic lateral sclerosis (ALS) patients have implicated Na+ and K+ channel dysfunction. It remains unknown, however, as to how these changes evolve longitudinally, and whether these changes are related to pathogenesis, neurodegeneration and clinical features. The objectives of the present study were to investigate longitudinal changes in axonal excitability and function in ALS patients and their relationship with axonal loss and clinical impairment. Axonal excitability studies were undertaken in 37 ALS patients (22 males; mean age, 53.5 years; SD, 10.2 years; median disease duration, 12.6 months; inter-quartile range, 9.5 - 26.4 months) at baseline and at 12 weeks follow-up. Measurements at baseline were compared to 39 healthy control subjects (mean age, 52.0 years; SD, 12.55 years). Functional impairment was evaluated using the ALS Functional Rating Scale-revised (ALS FRS-r). Patients were subdivided according to two mutually exclusive classification systems: 1) 10% reduction in peak compound muscle action potential amplitude; and 2) decline in fine motor subscore of ALS FRS-r. Baseline strength-duration time constant (a measure of nodal persistent Na+ conductance) was significantly prolonged in ALS patients (mean, 0.48ms; SD, 0.11ms) compared to healthy controls (mean, 0.44ms; SD, 0.11ms; $P = 0.02$), indicating increased entry of Na+ into the axons of ALS patients. By contrast, multiple measures of internodal K+ channel function were signicantly reduced at baseline. Longitudinal changes in all excitability variables implicated membrane hyperpolarisation as the primary phenomenon evolving in ALS patients. Furthermore, patients with preserved peak compound motor action potential amplitude demonstrated more severe changes in axonal excitability than those in whom compound potentials declined. Patients who reported progression in fine motor functional impairment generally recorded more severe changes in axonal excitability than those who did not experience decrement in fine motor function. Abnormalities in axonal function at baseline were consistent with increased nodal persistent Na+ conductances in ALS patients. Longitudinal studies suggested that peripheral nerves of ALS patients were becoming progressively

hyperpolarized. Axonal hyperpolarization may be a manifestation of increased firing of surviving motor units, compensating for neurogenic weakness.

P66 NEUROPHYSIOLOGICAL INDEX: EXPERIENCE WITH 142 ALS PATIENTS

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Keywords: neurophysiological index, M-wave amplitude, motor unit number estimation

Introduction: The neurophysiological index (NI) was described in 2000 by de Carvalho and Swash, as a simple and sensitive marker to measure lower motor (LMN) loss over ALS progression. Since then, other groups have applied this tool, in particular in clinical trials. We aim to review our experience on applying this marker in 142 patients with ALS from 2003 to 2010.

Methods: We analysed the mean percentage of change per months (%/m) for ALS-FRS, M-wave amplitude (ampl), MUNE and NI. MUNE was tested in 142 patients, 100 of whom with incremental technique (MUNE incr) and the last 24 with multipoint stimulation. This summarizes results published in 2003, 2005, 2006 and 2010, as well as new data (26 patients). The global results were adjusted for the number of patients included in each study.

Results: The mean age and gender distribution were not significantly different among studies, but disease duration showed large variation (mean of 22, 18, 16.4, 13 and 12 months, for studies 1 to 5, respectively). From study 1 to 5 (18, 39, 33, 28 and 24 patients, respectively), the mean change (%/m) was the following for ALS-FRS (3.57; 2.60; 2.02; 3.07; 2.90); ampl (3.42; 2.78; 5.04; 4.27; 3.22); NI (6.08; 4.48; 7.41; 6.99; 5.79); MUNE (not done; 4.70; 7.58; 6.74; 4.47). The variability (maximal - minimum value/mean) among results from different populations of ALS patients were 0.54, 0.60, 0.48, 0.53 (0.45 for MUNE incr) for ALS-FRS, ampl, NI and MUNE, respectively. We did not find any significant correlation between disease duration and rate of decline for the different measurements (Spearman's rho), or between ALS-FRS and the neurophysiological changes. The only significant correlation observed was between ampl and NI ($p < 0.001$).

Discussion: There is a small variation between among different groups of ALS patients regarding the rate of change of ALS-FRS, ampl, NI and MUNE, but NI and MUNE showed the smaller inter-group variation, indicating a good consistency of these markers. There was no correlation between disease duration and rate of change, suggesting that disease progression is not only determined by this factor. The strong correlation between ampl and NI represents that NI is derived from ampl. Our results support NI as a simple, sensitive and consistent marker of disease duration.

P67 COMPARISON OF MOTOR UNIT NUMBER ESTIMATION BY BAYESIAN STATISTICAL MUNE WITH HISTOLOGICAL COUNTING OF MOTOR UNIT NUMBERS: VALIDATION OF THE METHOD

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Keywords: MUNE, neurophysiology, biomarkers

Background: The number of motor units innervating a single muscle or a group of muscles determines muscle function. Motor unit number estimation techniques are commonly used to determine motor unit loss in ALS. We developed a method of motor unit number estimation, using neurophysiological data collected as the stimulus-response curve and Bayesian statistical analysis. Because this technique cannot be validated in humans, we tested this technique in a mouse model of ALS to compare the MUNE results with histological counts.

Objectives: Determine whether the number of motor units determined by Bayesian statistical MUNE is an accurate representation of the number motor units in a muscle, by comparing MUNE results with histological counts of motor neurons.

Methods: Male and female wild-type and SOD1G93A transgenic mice were studied at three stages of disease. Stimulus-response curves were obtained by application of a graded stimulus to the right sciatic nerve and collection of the compound muscle action potential from the right gastrocnemius muscle. Spinal cords were collected and motor neurones in the L4-5 spinal cord were counted by a physical fractionator method. Motor unit number was also calculated by our Bayesian MUNE method.

Results: In 10 presymptomatic wild-type animals, MUNE ranged from 40-54 and the number of motor units with histology ranged from 61-94. In 10 presymptomatic SOD1G93A mice, MUNE ranged from 27-53 and the number of units with histology ranged form 44-81. In 10 wild-type animals at disease onset age, MUNE ranged from 23-50 and histological counts ranged between 47-74. Onset SOD1G93A mice had a MUNE of 11-35 with histological counts ranging from 31-47. Ten wild-type animals at the end-stage age had MUNE ranging from 29-48 and histological counts from 53-97. By the end-stage of disease, SOD1G93A animals had much fewer motor units. MUNE ranged from 4-15 and histological counts ranged between 18-30 on 10 SOD1G93A end-stage animals. In all animals, the histology to MUNE ratio was approximately 2:1. Histological counts were highly correlated to MUNE ($p = 0.0168$ for wild-type and $p < 0.0001$ for SOD1). The progressive loss of motor units in SOD1G93A mice during disease progression occurred concurrently with denervation at the neuromuscular junction ($p < 0.001$ for all groups, $n = 5$ per group, t-test). There were no significant differences noted between male and female SOD1G93A mice.

Discussion and conclusions: Our MUNE method gives results that are approximately half of the number of anterior horn cells counted with histology. As our physiological method only measures functioning motor units, it is expected to be less than the anatomical count, which includes both functional and non-functional anterior horn cells.

P68 PRESYNAPTIC DECLINE OF THE NEUROMUSCULAR TRANSMISSION IN THE SOD1- G93A MOUSE MODEL OF ALS IS PREVENTED BY G-CSF IN GENDER-SPECIFIC MANNER

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Keywords: G-CSF, electrophysiology, neuromuscular junction

Background: Epidemiological studies show that both the incidence and prevalence of ALS are greater in men than in women. Sex differences have also been reported in mice overexpressing mutant SOD1, including the delayed onset in female G93A-SOD1 mice. However, the mechanisms underlying the role of gender differences in ALS are unknown. Because oxidative stress may be involved in motoneuron death in ALS and synapses are thought to be sensitive to oxidative stress, we characterized electrophysiologically the synaptic function in male and female G93A-SOD1 mice at early symptomatic stage of the disease.

Objectives: Our aim was to measure the synaptic properties of neuromuscular junction in diaphragm of an ALS mouse model and determine whether sex or anti-inflammatory treatment with granulocyte colony stimulating factor (G-CSF) affects the properties of synaptic transmission or level of reactive oxygen species (ROS) in spinal cord.

Methods: Transgenic mice overexpressing mutant Cu, Zn superoxide dismutase, G93A-SOD1, received weekly s.c. injections of G-CSF with sustained action, starting at presymptomatic stage. Properties of synaptic transmission and amount of ROS were measured from ALS mice at early symptomatic stage. Using intracellular microelectrode technique miniature and evoked endplate potentials (MEPPs and EPPs) were recorded in the diaphragm muscle and the amount of ROS was measured from the lumbar spinal cord.

Results: Electrophysiological testing revealed that the postsynaptic function was mainly preserved in G93A-SOD1 mice whereas the presynaptic properties were greatly affected by the disease. At postsynaptic site the membrane potential of muscle fibres was unaffected and postsynaptic sensitivity, measured as amplitude of MEPPs, was only slightly reduced in G93A-SOD1 mice. At presynaptic site, MEPPs were present in neuromuscular synapses of both sexes in wild type and transgenic mice. However, in transgenic mice the probability of spontaneous release was lowered and readily releasable transmitter pool was reduced when compared to those of wild type mice. In male G93A-SOD1 mice ROS reduced spontaneous quantal release, while female mice were resistant to this inhibitory action of ROS. The disease inflicted synaptic dysfunctions were improved by G-CSF treatment in male mice whereas the effect of the treatment was minor in females. Similarly, in the spinal cords of the G93A-SOD1 mice the level of ROS was increased in males but not in females and was reduced by G-CSF treatment.

Conclusions and discussion: This is the first detailed electrophysiological analysis of impaired synaptic function in a mouse model of ALS. In addition to the characterization of neuromuscular transmission mechanisms, we observed gender-specific prevention of synaptic impairment with anti-inflammatory G-CSF treatment.

P69 COL19A1 AS A POTENTIAL PROGNOSTIC BIOMARKER IN ALS

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Background: How skeletal muscle degenerates in ALS is currently unknown. However this tissue is one of the most promising targets for the search of potential biomarkers of ALS (1,2). In particular, one of the genes that is involved in regenerative pathways, to maintain the integrity of the tissue, is collagen type XIX, alpha 1 (*Col19a1*) (3). Due to the fact that regenerative processes tend to compensate for the induced unbalance under ALS degeneration, the possible role of *Col19a1* as a prognostic biomarker of ALS is under consideration.

Objectives: Our main goal is to study the transcriptional expression levels of *Col19a1* in skeletal muscle of transgenic $SOD1^{G93A}$ mice and in biopsy samples from ALS patients in order to analyze its role as a potential prognostic biomarker of longevity.

Methods: Muscle biopsies were carried out in forty eight $SOD1^{G93A}$ mice at each disease stage. Three biopsy samples were obtained per animal. Muscle biopsies from 8 ALS patients and 4 controls were obtained with prior informed consent. Gene expression variations in all samples were assayed by real-time PCR. Pearson correlation coefficient and Wilcoxon test were used in the statistical analysis.

Results: The longevity range in transgenic $SOD1^{G93A}$ varied from 105 to 160 days. The transcriptional levels of *Col19a1* varied significantly along disease progression and also correlated positively with longevity in $SOD1^{G93A}$ mice. The mean age in ALS patients (7 men and a woman) was 58.26 ± 5.59 years and 77 ± 6.4 years in controls (4 women). The transcriptional expression of *Col19a1* in ALS patients was significantly higher than in controls.

Discussion and conclusions: The significantly different gene expression profile and correlations of *Col19a1* found in transgenic $SOD1^{G93A}$ and in muscle biopsies of ALS patients and controls suggested that *Col19a1* could be considered as a potential prognostic biomarker of longevity in ALS. In neurodegenerative conditions such as ALS, *Col19a1* could compensate for the activation of degeneration signals in skeletal muscle, prompting muscle differentiation and regeneration. These results could shed light to find new biomarkers of ALS and finally a more accurate knowledge of the disease could be achieved.

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P70 NEUROTROPHIN RECEPTOR P75 AS A BIOMARKER FOR MOTOR NEURON DISEASE

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Keywords: biomarker, neurotrophin receptor p75 (p75NTR)

Background: There are no biochemical biomarkers for Motor Neuron Disease (MND), thus, finding a biological measure of the extent of this disease is critical. The American National Institute of Health defines a biomarker as 'a characteristic that can be measured and evaluated as an indicator of normal biological processes, pathological processes or pharmalogical responses to therapeutic intervention' (1). An important step in finding effective treatments is to identify biomarkers that could aid in the detection and progression of disease, and more rapid translation of potential therapeutics from models to clinical trials. The neurotrophin receptor p75 (p75NTR) is highly expressed during development and is greatly reduced in different types of cells in adulthood (2). However, multiple studies have found that p75NTR is robustly induced by injury (3). Of particular interest, is that p75NTR has been found upregulated in the spinal cord of: persons with MND postmortem (4) and the SOD1G93A mouse model of MND (5), and also in urine following sciatic nerve injury in rats (2).

Objectives: To determine if p75NTR is detectable in the urine of the MND mouse model SOD1G93A, and whether it could be used as a biomarker in the mouse model, allowing for more rapid translation of possible therapeutics to clinical trials.

Methods: SOD1G93A behavioural analysis was performed using neurological assessment, weight and hanging wire tests. The presence of p75NTR in SOD1G93A mice urine was detected with Western blot and confirmed by Immunoprecipitation.

Results: Behavioural testing showed that SOD1G93A mice ($n = 10$) developed motor symptoms at 120 days of age reaching end-stage by 150 days. p75NTR was detectable in SOD1G93A mice urine as early as 60 days of age, increasing with age, but was not detectable in age-matched controls ($n = 10$) until after 120 days.

Discussion: p75NTR was detectable pre-symptomatically in SOD1G93A mouse urine and may be useful as a biomarker of disease in the SOD1G93A model to allow for more rapid translation of potential therapeutics from the model to human clinical trials.

Conclusion: p75NTR has been identified as a possible biomarker for MND in the SOD1G93A mouse and on-going work is being undertaken to determine if it could be of use as a biomarker in human disease. Blood and urine samples from SOD1G93A mice and persons with MND will be tested for p75NTR by developing a more sensitive, quantitative ELISA assay.

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P71 DECREASED URINARY CONCENTRATIONS OF TYPE IV COLLAGEN IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: urine, serum, type IV collagen

Background: Recently, we have demonstrated that the basement membrane of skin in ALS patients was weakly positive for type IV collagen (IV-C) as compared with that of controls and that serum IV-C levels in patients with amyotrophic lateral sclerosis (ALS) were lower than in controls, suggesting that a metabolic alteration of IV-C may take place in the skin of ALS and that the decreased level of serum IV-C may reflect the decreased IV-C immunoreactivity of skin in ALS. We have postulated that measurement of urinary IV-C levels could be useful in assessing the alterations in basement membrane IV-C metabolism in the skin in ALS and for monitoring the progression of ALS.

Objectives: The aim of the present study was to measure the urinary concentrations of IV-C.

Methods: Our subjects were 20 patients with ALS(mean age $\pm$ SD, 57.6 ± 7.2 years; 14 men and 6 women), 20 diseased control subjects with other neurologic and muscular diseases (58.2 ± 9.1 years; 13 men and 7 women), and 20 age- and sex- mached healthy adults (51.4 ± 9.1 years, 12 men and 8 women). A polyethylene glycol (PEG)-based concentration method was used for concentrating urinary IV-C. Urinary IV-C concentrations were divided by urinary concentrations of creatinine to exclude the influence of various concentrations in urine. Urinary IV-C concentrations were measured by a one-step sandwich enzyme immunoassay (EIA).

Results: Urinary IV-C levels were 3.0 ± 1.5 ng/ml, 5.1 ± 2.0 ng/ml, and 5.7 ± 2.0 ng/ml in ALS patients, diseased control subjects, and healthy control subjects, respectively. The urinary level of IV-C was significantly lower in ALS patients than in diseased control subjects ($p < 0.001$) and healthy controls

($p < 0.001$). There was no appreciable difference in the urinary IV-C level between diseased control subjects and healthy controls. The urinary IV-C level was significantly and negatively correlated with duration of symptoms in patients with ALS ($r = -0.85$, $p < 0.001$), but there was no such correlation in diseased control subjects. No correlation was found between urinary concentrations of IV-C and dysphagia, muscle power rating ($r = -0.28$, $0.1 < p < 0.5$), severity of disability ($r = 0.30$, $0.1 < p < 0.5$) in ALS patients or diseased control subjects.

Discussion and conclusions: We used a 1-step sandwich EIA to measure urinary IV-C. Because this assay uses antibodies against both the 7S portion and the central triple helical portion, urinary IV-C measured by a sandwich EIA is a whole molecule and not a degradation product of preexisting IV-C. Measurement of decreased levels of urinary IV-C by this method probably reflects its decreased synthesis or increased degradation. Urinary concentrations of IV-C may be a sensitive indicator of altered IV-C metabolism and could also reflect basement membrane metabolism. These data suggest that a metabolic alteration of IV-C may occur in ALS patients.

P72 ELEVATED SERUM LEVELS OF AUTOANTIBODIES AGAINST HEAT SHOCK PROTEINS IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: autoantibody, heat shock proteins, biomarker

Background: Nowadays, the diagnosis of amyotrophic lateral sclerosis (ALS) is still mainly based on clinical symptoms and electromyographic findings. Hence a high rate of misdiagnosis and delays in proper diagnosis are almost unavoidable. Recent studies using proteomic approaches have identified a variety of potential serum biomarkers for the diagnosis of ALS. However, none of these has been proven to be effective for clinical use and further research is necessary.

Objectives: The expressions of heat shock proteins (HSPs) have been reported to be decreased before symptom onset and increased after symptom onset in brain tissue of G93A mice. These phenomena could be applied to develop potential biomarkers for ALS. HSPs belong to the damage associated molecular patterns (DAMPs), which are released by damaged cells, and could bind Toll-like receptors to activate inflammatory responses. To reduce inflammation, the immune system will produce autoantibodies to neutralize the inflammatory ligands, including HSPs. The aim of this study was to determine whether serum autoantibodies against HSPs could serve as surrogate biomarkers for the clinical diagnosis of ALS.

Methods: Forty ALS patients and 40 age-matched healthy adults were recruited. The ages of the patients were 32 to 86 y/o (mean = 61, SEM = 13), and that of the controls were 30 to 87 y/o (mean = 60, SEM = 17). The clinical severity of ALS was evaluated using the revised ALS functional rating scales (ALSFRS-R). The ALSFRS-R scores of our patients ranged from 0 to 33 (mean = 9.18, SEM = 10.22), and the disease duration ranged from 11.5 to 142.4 months (mean = 60.7, SEM = 38.5). The sera of patients and controlled subjects were collected. The serum autoantibodies against HSP60 and HSP70 were measured using enzyme linked-absorbent assay (ELISA). Student t-test was employed for statistics.

Results: Our results showed that the levels of serum autoantibodies against HSP60 and HSP70 were significantly higher (1.64- and 1.37-fold higher) in ALS patients as compared with those of age-matched controls. For the diagnosis of ALS, the performance of HSP60 autoantibodies showed 73% sensitivity, 74% specificity and 72% accuracy, and that of HSP70 autoantibodies showed 60% sensitivity, 60% specificity and 60% accuracy. The AUC values were 0.7651 and 0.6179 for autoantibodies against HSP60 and HSP70, respectively. Meanwhile, there is a tendency of increasing serum HSP60 and HSP70 autoantibody levels as disease severity increasing.

Conclusions: The elevated levels of HSPs have been reported in various neuronal injuries and neurodegenerative diseases. Our study provides a new idea that serum autoantibodies against HSP60 and HSP70 may be novel surrogate biomarkers for the diagnosis of ALS. This discovery also provided a clue to study the roles of serum autoantibodies in ALS.

P73 SERUM CREATININE (CR) TO CYSTATIN C (CYSC) RATIO (CR/CYSC) IS A GOOD SURROGATE MARKER OF RESIDUAL MUSCLE VOLUME IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: serum creatinine, serum cystatin C, surrogate marker

Background: The severity grade of ALS is approximately associated with the residual volume of skeletal muscles, and a parameter that reflects the muscle volume would enable us to estimate the severity. Since serum creatinine (Cr), though almost exclusively originating from skeletal muscle, is dependent on renal function, it cannot be such a marker in ALS patients. Cystatin (CysC), which is derived from all nucleated cells and independent of the body muscle volume, is excreted from the kidneys in the same way as Cr. Thus, the Cr/CysC ratio, which remains almost constant irrespective of the renal function in individuals without neuromuscular diseases, is supposed to be a good biomarker of the muscle volume, inverse of ALS severity.

Objective: To determine whether or not serum Cr/CysC may serve as a good surrogate marker of residual muscle volume, i.e., the inverse of the severity of ALS.

Methods: The serum levels of Cr and CysC were measured in 62 ALS patients admitted to our faculty during 2008-2010 (39 men, 23 women; mean age ± SD (years) = 62.9 ± 9.6) and 50 control subjects (mean age ± SD (years) = 62.8 ± 12.8). The correlation between Cr/CysC and severity grade of ALS (grades 1, 2, 3, 4 and 5 in ascending order of severity; the Research Committee of CNS Degenerative Diseases, the Ministry of Health, Labor and Welfare of Japan) was assessed.

Results: The Cr/CysC ratio was 7.90 ± 2.22 in the controls and 5.47 ± 3.31 in the ALS patients ($p < 0.05$). In ALS

patients, the means of serum Cr were 10.13, 7.82, 6.47, 6.30, and 2.37 ($F(4,56) = 18.19$, $p < 0.001$) for ALS severity grades 1, 2, 3, 4 and 5, Japanese people respectively. Meanwhile, the serum Cr/CysC are 1.01, 0.78, 0.64, 0.63, and 0.23 for the each severity grade respectively ($F(4,57) = 19.99$, $p < 0.001$). Post-hoc test with Tukey's HSD, we revealed significant differences in Cr/CysC between severity grades 1-4, 1-5, 2-5, 3-5, and 4-5 ($p < 0.05$).

Discussion and conclusions: In our study, Cr/CysC in ALS showed a clear inverse correlation with the disease severity, giving rise the possibility that it could be a renofunction-independent surrogate marker for assessment of the disease severity and progression. The ratio can be applied to various clinical trials in this condition.

P74 IDENTIFICATION OF A POTENTIAL SPORADIC ALS BIOMARKER IN CEREBROSPINAL FLUID

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Keywords: biomarkers, cerebrospinal fluid, Eph receptors

Background: The current lack of disease markers for sporadic ALS (sALS) has negative consequences for patients when it comes to clinical diagnosis, early medical intervention and participation in therapeutic trials. Valid biomarkers can be useful for diagnostic and prognostic indications as well as providing insight into disease pathogenesis and identifying targets for therapeutic interventions. Cerebrospinal fluid (CSF) may be a particularly valuable source of biomarkers because it is in close anatomical proximity to the brain and spinal cord, thus making it a better reflection of biochemical alterations resulting from neurodegenerative disease.

Objectives: We examined the proteome profile of CSF from sALS patients and controls to identify candidate biomarkers that could aid in the diagnosis of ALS and possibly provide insight into disease pathogenesis.

Methods: An antibody microarray technique was used to measure the expression levels of various cell signalling proteins in pooled CSF samples from sALS patients and healthy controls. Follow-up analysis by Western blotting with CSF from a separate cohort of sALS patients ($n = 19$), healthy controls ($n = 21$) and neurological disease controls ($n = 10$) were performed in order to validate protein changes observed in the microarray.

Results: Initial proteomic discovery studies revealed a decrease in the level of ephrin type-A receptor 1 tyrosine kinase (EphA1) in the pooled CSF of sALS patients relative to healthy controls. Independent validation studies with a separate cohort of sALS, healthy control and neurological disease control subjects confirmed that EphA1 expression in sALS CSF was significantly lower than both control groups. As a diagnostic test, EphA1 levels in CSF had a statistically significant ability to discriminate between sALS patients and controls. EphA1 levels also had a positive correlation with age at onset of sALS symptoms, indicating that this potential biomarker may be capable of identifying people who are prone to an earlier onset of disease.

Discussion and conclusions: The Eph family of tyrosine kinase receptors engage in complex bidirectional signalling that functions as a major form of contact-dependent communication between cells, and mediates a variety of cellular responses. As research begins to further elucidate these multifaceted signalling mechanisms, imbalances in Eph receptor functioning are increasingly implicated in a variety of pathologies in the central nervous system that are related to some of the major features of ALS. Larger scale studies are required to verify and further validate the candidate EphA1 biomarker, as well as define the functional implications of its down-regulation in sALS CSF. EphA1 should also be tested in conjunction with other potential CSF biomarkers to see if a more robust diagnostic test for ALS can be achieved. Finally, the levels of other receptors in the Eph family should be investigated and possible correlations with clinical parameters explored.

P75 BIOCHEMICAL ALTERATIONS ASSOCIATED WITH ALS

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Keywords: biomarker, metabolomics, pathophysiology

Background: There is no single diagnostic test for amyotrophic lateral sclerosis (ALS); imaging and laboratory tests are used to rule out diseases mimicking ALS. Electromyography can support an ALS diagnosis but is not specific. Average duration between symptom onset and diagnosis is 1 year. Metabolomics, the study of small molecules in biological systems is a potentially powerful technique to evaluate global biochemical alterations in a variety of diseases. Metabolomics could provide an understanding of the biochemical basis of ALS and identify biomarkers for diagnosis and disease progression. This could facilitate early diagnosis and trial entry, provide surrogate outcome measures, and decrease trial duration and size.

Objective: The plasma of ALS patients and healthy volunteers was analyzed for changes in the metabolic pathways in ALS patients manifesting as a uniquely altered metabolic signature.

Methods: Two cross-sectional biofluid collection studies were conducted. Plasma was collected from 62 (Study 1) and 99 (Study 2) ALS patients meeting El Escorial criteria for possible, probable, or definite ALS; 69 (Study 1) and 48 (Study 2) samples were collected from healthy volunteers**.** Unbiased metabolomic analysis was carried out on three independent instrument platforms: one gas chromatography/mass spectrometry (GC/MS) and two previously described ultrahigh performance liquid chromatography/tandem mass spectrometry (UHLC/MS/MS2) platforms optimized for either basic species or acidic species (1,2). Welch's two-sample t-test was employed to identify biochemicals with altered levels in ALS patients relative to healthy volunteer groups. For this hypothesis-generating analysis, biochemicals where $p < 0.05$ and $q < 0.2$ were considered statistically significant.

Results: A total of 335 biochemicals were identified in the plasma samples of the two studies. Of these metabolites,

240 (72%) were measured in both studies. In Study 1, ninety-two out of 282 (33%) metabolites were significantly altered in ALS patients, and in Study 2, fifty-five out of 293 (19%) were significantly altered. Twenty-three metabolites were significantly increased or decreased in both studies.

Discussion and conclusions: Using metabolomics, specific changes in the metabolic profile can be identified in patients with ALS relative to healthy volunteers. These changes point to potential disease mechanisms and potential therapeutic targets. The data presented here may provide insight into the pathophysiology of ALS and suggest promising areas for future studies. Metabolites involved in proposed ALS disease mechanisms were identified in the present analysis, including hypermetabolism, mitochondrial dysfunction, oxidative damage, hepatic dysfunction and neuronal breakdown.

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P76 BIOMARKER PROFILE DIFFERS BETWEEN CLINICALLY DEFINITE PRIMARY LATERAL SCLEROSIS (PLS) AND AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Keywords: PLS, biomarkers, oxidative stress

Background: PLS is clinically characterized by pure upper motor neuron dysfunction (PUMND) from onset throughout the disease course. Such criteria require that a diagnosis of PLS should not be made until at least 36 to 48 months after symptom onset. While long-term survival in PLS is common, it causes marked, progressive motor disability. To date, it is unknown if PLS is an independent disease or part of the MND spectrum. Recent neuroimaging and neurophysiological findings show unique features of PLS, but whether there are distinctive biochemical biomarker differences remains unknown.

Objectives: To determine if there are differences in oxidative stress (OS) biomarkers and lipid profiles in patients with PLS compared to those with ALS.

Methods: Overnight fasting urine and plasma specimens were collected from patients with clinically definite PLS (disease duration of at least 5 years but < 15 years and normal EMG done within 1 year of enrollment) in our on-going Spastic Paraplegia Foundation Multicenter PLS Study. Age- ($+5$ years) and sex-matched biosamples from patients with ALS (based on the El Escorial criteria and less than 18 months after symptom onset) were selected from the ALS Cohort Study of Multicenter Oxidative Stress (COSMOS) patient biobank. Lipid profiles were analyzed by standard lipid analyses ($n = 13$ both in ALS and PLS) and urinary OS markers were measured by immunoassay ($n = 15$ both in ALS and PLS).

Results: Total cholesterol and triglyceride levels were slightly higher in PLS (190 ± 18 and 129 ± 21 mg/dl, respectively) than ALS (151 ± 14 and 109 ± 16 mg/dl) but not statistically significant ($p = 0.1$ and 0.4, respectively). LDL was significantly higher in PLS (124 ± 14 mg/dl) than ALS (88 ± 10 mg/dl; $p = 0.05$). The ratio of LDL/HDL was higher in PLS ($3.2 + 1.4$) than ALS ($2.2 + 0.9$; $p = 0.06$). An OS biomarker, urinary isoprostanes (lipid oxidation products), was not different, but levels of urinary 8-oxodeoxyguanosine (oxidized DNA base) were significantly elevated in ALS ($28 + 11$ nmol/mmol) compared to PLS ($21 + 9$ nmol/mmol; $p = 0.06$).

Discussion and conclusions: Our study suggests some biochemical differences between PLS and ALS despite the small sample size. An increased ratio of LDL/HDL has been associated with longer survival or with less respiratory distress in patients with ALS. Our findings are consistent with the less respiratory distress and longer survival of PLS patients. Further, the findings suggest lower levels of oxidative stress among PLS cases. This is the first demonstration of biochemical differences between PLS and ALS. Further studies are clearly needed to elucidate the progression of biomarkers in the natural history of PLS. (The study is funded by R01-ES016348, MDA, and Spastic Paraplegia Foundation).

P77 TOWARDS DEVELOPING A LONGITUDINAL WHOLE-BRAIN PROTON-MR METABOLITE IMAGING DATABASE FOR EVALUATING TEMPORAL CHANGES OF METABOLITES IN ALS

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Keywords: biomarkers, brain, spectroscopy

Background: Brain metabolite concentrations as measured by proton MRS are known to be altered in several pathologies including ALS. Previous brain-MRS studies in ALS have acquired data from predetermined single-voxel and multi-voxel locations that resulted in assessment of metabolite changes from limited brain regions. The accumulating evidence for extramotor involvement warrants evaluation of metabolite level alterations from the whole brain. In this study, a whole-brain MRSI acquisition and fully-automated data processing and unbiased analyses methods were used to evaluate metabolite changes longitudinally.

Objectives: To develop a multi-time point whole-brain proton-MR metabolite imaging database for evaluating temporal changes in metabolite concentrations and to assess its use for longitudinal studies.

Methods: Seven subjects with definite sporadic-ALS were scanned at 3T, 2-times with intervals of 4-to-10 months for 5 and 19, 29 months for 2 subjects. The whole-brain MRSI were obtained using a volumetric acquisition sequence with TR/TE of 1710/70. The metabolites, N-acetyl aspartate (NAA), total-creatine (Cre), total-choline (Cho) and Cho/NAA were quantified using 43-region and 9-region (8 hemispheric lobes + cerebellum) anatomical atlases. Lobar analysis included calculating values for gray-matter (GM) and white-matter (WM).

Comparison of metabolite measures between the 2-times were performed using the 2-sided paired t-test. A p-value of < 0.05 was considered significant.

Results: Using the lobar-atlas, significant metabolite changes were observed in ALS for NAA in the GM of bilateral temporal and left parietal, for Cho in the GM of right temporal, for Cre in the WM of frontal and right temporal lobes. Changes approaching towards significance were observed in left occipital WM for Cre, and left-frontal GM for Cho/NAA. Of the 9 regions, significant changes were observed from 5 regions for at least one of the metabolites and/or tissue-types.

Using the 43-region-atlas, significant metabolite changes were observed in ALS for the following regions: left-occipital, bilateral-parietal, right-paracentral-lobule, bilateral-caudate, bilateral-thalamus and left-temporal for NAA; left-frontal, left-occipital, left-fusiform, right-parietal, right-paracentral lobule, left-caudate, right-pallidum, bilateral-thalamus and bilateral-temporal for Cre; right-postcentral, right-paracentral lobule and right-thalamus for Cho; and right-postcentral for Cho/NAA. The p-values for several other regions showed approach towards significance. In all, 14 of the 43 regions showed significant changes for at least one of the metabolites.

Discussion and conclusions: The observation of significant metabolite changes temporally from several lobar and anatomical substructures of ALS patients indicate that the changes are anatomically widespread. Whole-brain MRSI acquisition method and its unbiased data analyses approach are well-suited for evaluating longitudinal changes in metabolites. Further studies with data from additional number of subjects and time-points are required to assess use of this data for assessing efficacy of drugs.

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P78 WHOLE-BRAIN PROTON MR METABOLITE IMAGING OF ALS AND CORRELATION WITH CLINICAL ASSESSMENTS

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Keywords: biomarkers, brain, spectroscopy

Background: Brain metabolite changes in ALS were evaluated mostly in the motor cortex (MC) and corticospinal tracts (CSTs) using single-voxel and 2D-MR spectroscopic imaging (MRSI) methods. Such methods are inherently biased as only data from predetermined anatomical regions are acquired. In this study, a whole-brain MRSI acquisition and fully-automated processing and unbiased analyses methods were used to evaluate metabolite changes.

Objectives: To evaluate metabolite alterations in whole-brain MRSI by performing data analyses by tissue-types (white-matter (WM) and gray-matter (GM)), lobes and whole-CSTs, and correlate that with clinical assessments in ALS.

Methods: Thirty-eight subjects with definite sporadic-ALS and 70 age-matched controls were scanned at 3T. Clinical measurements included ALSFRS-R, vital capacity and assessments of upper motor neuron (UMN) function. The whole-brain MRSI were obtained using a volumetric acquisition sequence with TR/TE of 1710/70 ms (1). The metabolites, N-acetyl aspartate (NAA), and Choline (Cho)/NAA were quantified by brain lobes (frontal, temporal, parietal and occipital) and tissue-types and by whole-CSTs, bilaterally. Comparison of metabolite measures between the groups were performed using ANCOVA with age as a covariate, and associations between the metabolite and clinical measures were evaluated using Pearson's product moment partial correlation. A p-value of < 0.05 was considered significant.

Results: In the whole-CSTs of ALS: Highly significant changes (p: 0.005 to $< <$ 0.0002) found for the metabolites in both the sides were: 1) 7.5% and 4.6% decrease for NAA in the left and right, respectively; 2) 19.6% and 15.1% increase for Cho/NAA for the left and right, respectively. The NAA and Cho/NAA showed significant correlations with the rate of finger taps (NAA-left: $r = 0.58$, $p = 0.0001$; NAA-right: $r = 0.40$, $p = 0.01$; Cho/NAA-left: $r = -0.546$, $p = 0.0001$; Cho/NAA-right: $r = -0.470$, $p = 0.003$).

In the lobes of ALS: In the WM, NAA decreased by 6.0 % in frontal and 3.1% in parietal, and Cho/NAA increased by 10.1% in frontal, 5.4% in parietal, and 6.2% in occipital. For the GM, NAA decreased by 3.5% only in right-frontal, and Cho/NAA increased by 6.75% in frontal. The rate of finger taps correlated with NAA in the frontal WM ($r = 0.348$, $p = 0.03$) and GM ($r = 0.349$, $p = 0.03$) and with Cho/NAA in the WM ($r = -0.329$, $p = 0.04$).

Discussion and conclusions: The observed significant metabolite changes from the frontal, parietal and occipital lobes indicate that the ALS pathology is not anatomically localized only to the MC and CSTs but is more widespread. Significant correlations between NAA in the frontal lobe and CSTs and the rate of finger tapping support use of NAA as a biomarker of UMN dysfunction. Whole-brain MRSI and its unbiased metabolite analyses are shown to be well-suited for evaluating ALS pathology.

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P79 CORTICAL IMAGING: AN UPPER MOTOR NEURON MARKER FOR AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: MRI, neuroimaging, cortical thickness

Background: Upper motor neuron involvement for the diagnosis of amyotrophic lateral sclerosis (ALS) can be difficult to establish. An objective upper motor neuron marker would facilitate diagnosis. MR imaging is a widely available tool to study the brain anatomy. Computational techniques allow for detecting subtle degenerative effects on the brain.

Objectives: To study the degenerative effects of ALS on the morphology of the whole cortical mantle with an unbiased surface based approach.

Methods: We performed surface based cortical morphology analyses on structural, 3T MRI data of 45 patients with ALS and 25 age and gender matched healthy controls. Cortical morphology analyses consisted of measuring cortical thickness, surface area and volume. The effects of disease progression

were examined by longitudinal measures in a subset of patients and correlating cortical measures with progression rate.

Results: Cortical morphology analyses revealed specific thinning in the precentral gyrus, being the primary motor cortex, in patients with ALS compared to controls ($p = 6.3\times10^{-8}$). Individual measures of the cortical thickness in the precentral gyrus discriminated patients with ALS from the healthy controls with a specificity of 82% and a sensitivity of 84%. Regarding the three morphology measures (thickness, surface area and volume), cortical thickness was found superior in detecting the degenerative effects of ALS. Relative cortical thinning in temporal regions was related with a faster disease progression ($p = 3.3\times10^{-4}$).

Conclusions: Cortical thinning of the primary motor cortex is a sensitive marker for upper motor neuron degeneration, proposing cortical imaging as a promising diagnostic marker for ALS. Progression of disease did not reveal additional cortical thinning in the primary motor cortex, suggesting these effects are potentially present before clinical diagnosis. Relative cortical thinning in temporal regions, however, was associated with a more progressive disease course.

P80 INCREASED 18F-FDG UPTAKE IN SUB-CORTICAL STRUCTURES OF ALS PATIENTS

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Keywords: 18-F FDG PET/TC, sub-cortical areas, metabolic increase

Background: Recent data suggest that ALS is a multisystem disorder, characterized by the involvement of various central nervous system areas besides the motor cortex.

Aim: To assess PET/CT brain metabolic changes in a series of consecutive ALS patients.

Methods: ^{18}F-FDG PET/CT scans of 32 ALS patients (13 bulbar, 19 spinal) were compared to those from 22 healthy controls. Differences were analyzed by statistical parametric mapping (SPM2), controlled by age and gender. A $p < 0.05$ threshold was used for SPM t-maps, corrected for multiple comparisons with the False Discovery Rate (FDR) option at voxel level and $p < 0.001$ corrected for multiple comparison at cluster level.

Results: Compared to controls, ALS patients showed a significant lower FDG uptake in the right ® and left (L) premotorcortex (Brodmann Area (BA) 6), R and L lingual gyrus (BA 18), R primary visual cortex (BA 17), R fusiform gyrus (BA 18) and L pre-central gyrus (BA 8, 9). The reverse comparison resulted in higher FDG uptake in ALS patients in the R and L amygdala, R and L pons and midbrain, R and L cerebellar tonsil, R lateral globus pallidus. ALS patients showed also a highly significant increase in FDG uptake in the brainstem (subthalamic nucleus, substantia nigra, and red nucleus). Bulbar onset patients showed high FDG uptake in R and L pons, and low uptake in almost all fronto-temporal regions; in spinal patients, FDG uptake was higher in R and L midbrain (BA 21, 28), and was reduced in R and L lingual gyrus (BA 18) and R fusiform gyrus (BA 19).

Conclusions: This PET/CT study revealed increased ^{18}F-FDG uptake in large subcortical and brainstem regions in ALS. Significant differences between bulbar and spinal onset cases were also found. We hypothesize that the increase of glucose metabolism may be related to a reactive astrocytosis and/or microgliosis.

P81 DIFFUSION TENSOR IMAGING (DTI) MEASURES IN THE CORTICOSPINAL TRACTS AND THEIR CORRELATIONS WITH UPPER MOTOR NEURON FUNCTION MEASURES IN PATIENTS WITH ALS

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Background: Regions-of-interest (ROI) analyses of diffusion tensor imaging data have shown a reduction in fractional anisotropy (FA) along the corticospinal tracts (CST). However, its correlation with motor disability and clinical measures of upper motor neuron (UMN) function is still not clear or well established to be incorporated into diagnostic criteria.

Objective: To evaluate association between a DTI measure, fractional anisotropy (FA) and clinical measures of UMN function.

Design and methods: Fifty-one ALS patients (49 definite, 2 probable; 43 limb onset; 53 ± 9 years, disease duration: 19 ± 10 months) and 49 age-matched controls (51 ± 9 years) had evaluation for upper motor neuron function (maximum finger-and foot tap rates, *pa-pa*-and *la-la* syllable repeat rates; UMN burden score based on definite UMN signs, such as Hoffmann sign, Babinski sign and pathologic muscle stretch reflexes or spasticity). The disease severity was assessed using ALSFRS-R and vital capacity. Subgroup of subjects, 15 definite ALS- patients and 14 age matched controls, had whole brain scan on a 3T scanner using a spin-echo based DTI sequence (TR/TE = 6400/87 ms, b = 0, 1000 s/mm2, 12 gradient directions, 34 slices, 3 mm thickness). DTI data were processed using DtiStudio (https://www.mristudio.org/), and 3 ROIs were drawn. These included the posterior limb of the internal capsule (ROI-1), the midbrain at the level of the cerebral peduncle (ROI-@) and at the mid-pons (ROI-3), and FA values were obtained.

Results: The important findings were: the mean-FA was lower in patients with ALS than controls in all ROIs, bilaterally, within the CSTs (right-ROI-1: 0.60 ± 0.06 vs 0.66 ± 0.03, $p = 0.003$; right-ROI-2:0. 65 ± 0.05 vs 0.71 ± 0.04, $p = 0.001$; right-ROI-3:0. 51 ± 0.06 vs 0.61 ± 0.06, $p = 0.0001$). The maximum finger tap rate (3.4 ± 0.9 vs 4.1 ± 0.5; $p = 0.0001$), foot tap rate (2.2 ± 1.1 vs 3.4 ± 0.4; $p = 0.0001$), *pa pa* syllable repeat rat ($3.4 \pm 0.0.9$ vs 4.1 ± 0.5; $p = 0.0001$) and *la la* syllable repeat rate (3.3 ± 1.0 vs 4.1 ± 0.4; $p = 0.0001$) were significantly lower in ALS patients than controls. There were

mild to strong positive correlations between FA at various levels along the CST and clinical measures of UMN function in all the subjects ($r = 0.4 - 0.8$) except the UMN burden score, which correlated poorly with FA from all the ROIs ($r = 0.2$-0.4). ALSFRS-R poorly correlated with FA from all the ROIs ($r = 0.2$-0.5).

Conclusions and relevance: The reduced-FA reflects the functional abnormality of CST and its positive correlation with UMN function measures suggests that it can be used to assess UMN involvement in ALS patients objectively, and it may therefore contribute to earlier diagnosis of the disease.

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P82 WHOLE-BRAIN DTI PATTERN OF WHITE MATTER DAMAGE IN AMYOTROPHIC LATERAL SCLEROSIS: FURTHER EVIDENCE OF A MULTISYSTEM DISORDER

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Keywords: diffusion tensor imaging (DTI), tract based spatial statistics (TBSS), axonal injury

Background: Even if nowadays amyotrophic lateral sclerosis (ALS) is not anymore considered a basically motor disease, the actual spread of the neurodegenerative process throughout the central nervous system is not fully understood. In this work, we performed whole-brain tract-based spatial statistics (TBSS) and volume-of-interest (VOI) diffusion tensor magnetic resonance imaging (DTI-MRI) analyses to detect white matter (WM) patterns of microstructural abnormalities in ALS, and to correlate the DTI parameters with clinical indices of disability and pyramidal impairment.

Methods: Brain MRI and DTI were performed at 3 Tesla on 19 ALS patients with upper motor neuron (UMN) score (a clinical measure of pyramidal impairment) ranging from 2 to 16 and disease duration (DD) ranging from 1 to 14 years, in comparison to 20 age- and sex-matched healthy volunteers. Group-level analyses of DTI data sets were carried out with the Functional MRI of the Brain (FMRIB) Software Library (FSL) software package.

Results: Compared with controls, ALS patients showed a significant decrease in the fractional anisotropy (FA) in the body of corpus callosum (CC) ($p < 0.05$, corrected). When analyzing the ALS group alone, at the VOI level we observed both FA decrease and radial diffusivity (RD) increase in the body of CC that were significantly correlated with UMN score ($p = 0.003$ and $p = 0.02$). In addition, significant voxelwise positive correlations between FA and ALS functional rating scale revised (ALSFRS-R) score (index of patients' disability) were detected in the WM tracts underneath the left premotor cortex ($p < 0.05$, corrected).

Discussion and conclusions: The correlations between reduction of FA and increase of RD in the body of CC proportional to the UMN score suggest that the amount of WM degeneration in the CC is strictly related to ALS pyramidal impairment, and mainly determined by axonal loss within the motor fibers pathway. The correlation between FA and ALSFRS-R in the associative tracts underneath left premotor cortex might reflect the progressive spread of disease from motor towards extra-motor areas.

P83 VARIATE DIFFUSION TENSOR IMAGING ANALYSES FOR CHARACTERIZATION OF MOTOR NEURON DISEASES

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Keywords: diffusion tensor imaging, tractwise fractional anisotropy statistics, variate analysis

Background: Pathophysiological processes in MND are related to the predominance of upper or lower motor neuron affection. Novel approaches based on computer-based techniques such as diffusion tensor imaging (DTI) are able to image disorders within the cerebral white matter (WM) *in vivo.* In a broad variety of studies, DTI has proven to be a valuable tool for analyzing these WM alterations.

Objectives: The objective of this study was to investigate patterns of CST and CC degeneration in different MND with involvement of the upper motor neuron by application of specific DTI analysis approaches.

Methods: Seventy-two patients with MND, i.e. amyotrophic lateral sclerosis (ALS, $N = 20$), primary lateral sclerosis (PLS, $N = 20$), hereditary spastic paraparesis (HSP), subdivided into pure HSP (pHSP), $N = 20$ and complicated HSP (cHSP), $N = 12$, were analyzed by application of variate DTI analyses in comparison with matched controls, in order to identify differences in FA. All analyses were performed by the Tensor Imaging and Fiber Tracking (TIFT) software (1). Analysis was performed in a variate fashion, i.e. voxelwise comparison of FA maps at the group level, volume of interest (VOI) analysis, as well as fiber tracking (FT) on group averaged data accompanied by tractwise fractional anisotropy statistics (TFAS) (2).

Results: The variate analyses of WM impairment demonstrated characteristic patterns of widespread alterations within the motor system for the various MNDs (3). A predominant affectation of the corticospinal tract (CST) and also WM changes within distinct areas of the corpus callosum (CC) were observed. In detail, the PLS group showed significant FA reductions in motor regions of the CC while the alterations of the pHSP group exceeded the motor region into the splenium and the changes of the ALS group to rostral parts of the corpus, respectively. Finally, the cHSP group showed significantly reduced FA within the whole CC and extensive areas of the subcortical WM.

Discussion and conclusions: In summary, variate DTI analysis was useful in order to define a distinct WM pathoanatomy in specific brain areas involving the CST and the motor segment (III) of the CC.

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P84 VOXEL-BASED RELAXOMETRY OF T2 MAPS IN THE BRAIN OF AMYOTROPHIC LATERAL SCLERORSIS

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Keywords: voxel-based relaxometry, magnetic resonance imaging, T2 relaxometry

Background: Relaxation describes the processes that spin magnetization prepared in a non-equilibrium state returns to the equilibrium distribution in magnetic resonance imaging. Relaxometry refers to the measurement of relaxation magnetic resonance imaging. Voxel-based relaxation is more sensitive to changes of tissue properties, i.e. differences in relaxation rates for instance increased iron deposition.

Objectives: To evaluate the characteristics of T2 relaxometry in the brain of amyotrophic lateral sclerorsis (ALS) patients using voxel-based relaxometry (VBR).

Methods: 32 definite or probable ALS patients based on E1 Escorial standards and 32 healthy controls were recruited and underwent a neuropsychological evaluation. The T2 mapping sequence data was collected on a GE Medical 3.0T MRI system. ANCOVA was applied with age as a covariate because of an exact sex match. A statistical threshold of $P < 0.001$ (uncorrected, $t > 3.23$) and more than continuous 20 voxels determined significance. The correlation analysis was applied between the mean intensity of active clusters and the progression scores by SPSS 15.0 and the curve fitting toolbox of matlab (version 7.6.0.324).

Results: VBR identified the T2 values increase in the right superior temporal gyrus white matter and inferior parietal lobule gray matter, the left superior frontal gyrus white matter and inferior frontal gyrus gray matter ($P < 0.05$). No areas of statistically significant decrease was displayed.

Discussion and conclusions: Voxel-based relaxometry demonstatred the good assessment of T2 value differences through the brain in ALS, and it could be regarded as an alternative means of relaxometry data analysis to evaluate the iron deposition in the brain of ALS.

P85 IS THERE A DISTINCT CEREBRAL PATHOLOGY IN PLS? EXPERIENCE WITH A NOVEL MYELIN-FOCUSED MRI RELAXOMETRY SEQUENCE

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Keywords: biomarker, myelin, inflammation

Background: Cerebral Wallerian demyelination and related neuroinflammatory processes have not been systematically studied in motor neuron diseases. Multi-component Driven Equilibrium Single Pulse Observation of T1 and T2 (mcDESPOT) is a novel MRI relaxometry technique allowing assessment of the myelin water fraction (MWF - a measure related to myelin content), and estimation of the myelin intra/extracellular (IE) water T2 properties linked to the presence of inflammatory processes.

Objectives: To use mcDESPOT as a marker of demyelination and inflammatory processes in heterogeneous cases of ALS, a group of definite PLS patients, and a group of healthy controls.

Methods: Data were acquired at 3T. Eighteen ALS patients of variable upper motor neuron (UMN) involvement clinically, 7 definite PLS patients and 9 healthy controls were included in the study. Image processing was performed to derive voxel-wise MWF and IE-water T2 maps. A WM skeleton was created from the MWF maps using tract-based spatial statistics (TBSS). Voxel-wise non-parametric testing was performed on the skeletonised MWF and IE-water T2 data to determine areas of group difference (ALS-control; PLS-control; ALS-PLS).

Results: Reduced MWF and increased IE-water T2 was found in both patient groups. MWF was able to discriminate the PLS group from controls, where widespread and symmetrical regions of decreased MWF were noted throughout the WM skeleton (average 4.8% decrease, and 2.6% across the entire WM skeleton; both $p < 0.05$), with areas of increased IE-water T2 in parts of the descending corticospinal tract (CST) and also corpus callosum (mean 5.2% increase in areas denoted in figure 1; 1.4% across the entire WM skeleton; both $p < 0.05$). In the ALS group, the MWF was not significantly decreased compared to controls (mean 0.7% lower across the WM skeleton), including in a sub-group analysis of just those with very high UMN scores, and those with a disease duration of more than 5 years. There were regions (largely motor) where MWF was significantly lower for PLS patients compared to ALS (regional mean 4.8% lower in PLS, 1.8% across the WM skeleton), with more widespread increased IE-water T2 (mean 3.7% increase in significant areas, 0.8% for the entire WM skeleton). There was no correlation between imaging parameters and disability for either group.

Discussion: mcDESPOT revealed a distinct pattern of reduced MWF in PLS compared to ALS patients, that appeared to transcend both UMN burden of disease clinically and disease duration, and suggests widespread demyelination. There was also indirect evidence of inflammatory activity from the increased regional IE-water T2 found in both patient groups, which may have implications for therapeutic intervention as well as understanding prognostic heterogeneity.

Conclusions: mcDESPOT has the potential to reveal novel aspects of *in vivo* pathology non-invasively, and adds to the growing biomarker potential of advanced MRI applied to motor neuron diseases.

P86 STRUCTURAL AND FUNCTIONAL CONNECTIVITY APPEAR INVERSELY RELATED WITHIN THE ALS CEREBRAL NETWORK

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Keywords: network, cerebral connectivity, biomarker

Background: ALS as a system failure is a concept based on two decades of research confirming a consistent extra-motor CNS pathology, and supported by recent functional MRI studies revealing altered resting-state networks (1). There is a need to understand the functional alterations associated with structural changes detected using advanced MRI techniques such as diffusion tensor imaging (DTI, for investigating white matter tract integrity) and voxel-based morphometry (for detecting grey matter volume changes).

Objectives: To characterize the functional connectivity changes within the ALS-specific cerebral network.

Methods: This was a cross-sectional multimodal MRI study in a heterogeneous group of ALS patients (n = 26) recruited from the ongoing longitudinal Oxford Study for Biomarkers in MND. Patients were compared to age and gender-matched healthy control subjects (n = 15). All subjects underwent high-field (3T) T1-weighted (1 mm isotropic), DTI (2 mm isotropic, 60 directions,), and resting-state functional MRI (3 mm isotropic). Tract-based spatial statistics (TBSS) on the DTI data characterized the white matter regions significantly altered in the ALS group. Tractography was used to identify grey matter regions linked to this impaired white matter network. A dual-regression analysis of the whole-brain resting-state functional MRI data was performed using this ALS-specific tractography-derived structural network to determine any modified functional connectivity in relation to abnormal structural connectivity.

Results: TBSS confirmed bilateral corticospinal and corpus callosum white matter tract involvement (2). Associated grey matter regions included motor, premotor and supplementary motor cortices, pars opercularis and motor-related thalamic nuclei. A spatial pattern of increased functional connectivity matched the pattern of decreased structural connectivity in all grey matter motor-related areas. Patients with longer disease duration showed relatively higher white matter tract integrity but lower functional connectivity, with values closer to those of the healthy controls in all instances. A composite measure of grey matter volume, structural connectivity functional connectivity information was ~95% accurate for the discrimination of ALS patients.

Discussion: A combined structural and functional approach identified dichotomous processes characterizing the ALS cerebral network failure, involving increased functional connectivity within regions of decreased structural connectivity. Whilst this combination might reflect a compensatory response, an upstream dysregulation driving pathogenesis is plausible, possibly arising through loss of inhibitory neuronal influences (an 'interneuronopathy') given the similarity in regional loss of [11C]-flumazenil binding noted in a PET study (3).

Conclusions: Integrating multimodal MRI reveals the ALS cerebral signature in vivo and may generate surrogate markers of disease activity. Longitudinal, combined structural and functional studies, including the study of pre-symptomatic carriers of pathogenic ALS-related gene mutations, may ultimately allow the development of a Braak-like in vivo model of ALS pathogenesis.

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P87 DIRECT EVIDENCE OF INTRA- AND INTER-HEMISPHERIC CORTICOMOTOR NETWORK DEGENERATION IN ALS: AN AUTOMATED MRI STRUCTURAL CONNECTIVITY STUDY

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Keywords: MRI, tractography, connectivity

Background: There is a growing interest in developing non-invasive neuroimaging biomarkers that can be used to improve our understanding of the pathogeneses of ALS and provide improved measures of disease progress. We have developed a fully automated technique for measuring the structural connectivity of corticomotor white matter (WM) pathways, based on diffusion MRI tractography in conjunction with cortical parcellation of high resolution structural images, that is optimised for resolving fibres within complex WM architecture (1).

Objectives: Our hypothesis is that quantitative intra- and inter-hemispheric structural connectivity measures based on assessing the Fractional Anisotropy (FA) for each corticomotor pathway will provide new insight into corticomotoneuron involvement in ALS. The reproducibility of the corticomotor connections were also assessed in control participants.

Methods: Structural MRI and HARDI diffusion data (64 diffusion encoding directions, b = 3000 mm/s^2) were acquired from 15 ALS patients with mixed upper and lower motor neuron signs and 18 controls using a 3T scanner. Connectomes were generated using our automated pipeline (1). Each element within the connectivity matrix (i.e. cortical connection) was encoded with the mean FA value along that trajectory. Statistically significant differences in corticomotor connectivity between the ALS and control group were detected by applying a nonparametric Mann-Whitney test (applying a FDR of 10%). Eight control subjects were scanned twice over a period of 6 months to assess reproducibility.

Results: The reproducibility of the corticomotor connections was high (within 5%). In ALS patients there was significant loss in connectivity within a number of corticomotor pathways. With regards to altered intrahemispheric connectivity, there was a significant reduction in FA within the corticospinal connections of the brainstem (bs) with left precentral gyrus (lh.preCG), bs and right precentral gyrus (rh.preCG), bs and right postcentral gyrus (rh.postCG). Other intrahemispheric pathways significantly involved with ALS pathology were connections between the lh.preCG and left posterior cingulate (lh.postC), the rh.postCG and right paracentral gyrus (rh.paraCG), and the rh.postCG and right posterior cingulate (rh.postC). Novel results were also found for a number of interhemispheric pathways including the lh.preCG

and rh.preCG, the lh.preCG and rh.postCG, and lh.preCG and right superior frontal gyrus (rh.supFG).

Discussion: The reduced connectivity associated with the precentral and postcentral gyri is consistent with known pathology and highlights the spread of neuropathology along multiple corticomotor pathways in ALS and supports a mechanism involving anterograde corticomotor neuron degeneration (2) in patients with mixed upper and lower motor neuron signs.

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P88 CONTRIBUTION OF SPINAL MRI IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: MRI, sensory pathways, spinal cord

Background: Amyotrophic lateral sclerosis (ALS) is a fatal disease characterized by upper (i.e. corticospinal tract, CST) and lower motor neuron degeneration. However, histopathological studies have shown involvement of spinal sensory pathways, supported by more recent *in vivo* imaging of the brain. These findings bring new insights to the pathogenesis of ALS. Characterizing *in vivo* spinal lesions in ALS is crucial to explore the anatomical structures affected by ALS. Novel MRI techniques such as diffusion-weighted (DW) and magnetization transfer (MT) imaging provide sensitive markers of white matter pathology.

Objectives: To combine DW and MT imaging of the cervical spinal cord in ALS patients and assess the presence of CST and dorsal column degeneration.

Methods: Patients with ALS (N = 29, Mean age = 53 ± 10 years, Median disease duration = 1.4 years) and age-matched controls (N = 21) were recruited. Patients were clinically assessed and scored on the ALS Functional Rating Scale (ALSFRS-R) and motor evoked potentials (MEP) were obtained using Transcranial Magnetic Stimulation (TMS). Subjects were scanned at 3T with DW and MT imaging. Manual ROIs were drawn in the spinal cord to isolate lateral (containing the CST) and dorsal (containing sensory afferents) segments. DTI and MT metrics were quantified in both ROIs and compared between patients and controls, then correlations with clinical scores and TMS measures were computed.

Discussion and conclusions: In the lateral region (CST), significant differences were detected between patients and controls for FA ($p < 0.001$), radial diffusivity ($p < 0.001$) and MTR ($p < 0.01$). No significant difference was detected for axial diffusivity ($p = 0.15$) and MD ($p = 0.05$). More interestingly, significant differences were detected in the dorsal column for FA ($p < 0.001$), radial diffusivity ($p < 0.01$) and MTR ($p = 0.02$). Pearson's coefficient showed significant correlations between FA in the lateral (CST) region and ALS-FRS-R ($p = 0.04$) and TMS motor threshold ($p = 0.02$).

P89 ALS AND BODY MOVEMENTS – COMPENSATION IN HIGHER ORDER PROCESSING AREAS

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Keywords: cortical plasticity, movement imagery, fMRI

Background: Execution, imagery, and perception of movements share similar neuronal substrates. A hallmark of ALS is progressive neuronal loss in primary motor areas. In previous studies, ALS patients showed more activity in higher order motor networks than healthy controls during execution and imagery of simple hand movements suggesting compensatory processes.

Objectives: In the present study we investigated if such compensatory neuronal processes can be seen for imagery and perception of more complex body movements of everyday life.

Methods: In this study, 7 ALS patients and 14 healthy controls were investigated using functional magnetic resonance imaging (fMRI).Thirteen movements were used for kinesthetic movement imagery and visual perception: four isolated movements (e. g., writing), four body related movements (e. g., tooth brushing), four movements which can be performed also in later stage ALS (e. g., blinking), and one control movement (rolling ball). Before fMRI measurement, subjects practised the kinesthetic imagery of each body movement and the visual imagery of the rolling ball; surface EMG electrode recording was performed to monitor possible subtle muscular activity during imagery. Additionally, motor impairment of the patients was assessed using the ALS functional rating scale revised (ALS-FRS-R). Several cognitive and psychiatric measurements were performed with all subjects.

Results: Preliminary data analysis suggests similar activation in ALS patients and controls during movement perception mainly in extrastriate visual areas (e. g., V2, V3), areas involved in higher order visual processing (e. g., MT), and premotor areas (BA 6). Patients showed more cortical activity during movement perception than healthy controls in areas for higher order movement representation (e. g., BA 40), whereas controls showed higher activity mainly in the right premotor cortex.

During movement imagery both groups similarly activated premotor areas, a network known as mirror neuron system (BA 44, BA 45), and higher order visual areas (e. g., BA7). ALS patients showed more activity in the premotor cortex than controls during this task. In contrast, healthy controls showed higher activity than patients in subcortical (e. g., putamen) and cortical (e. g., hippocampus) structures related to motor mem-

ory. The more advanced the disease, the stronger the activity in areas of higher order movement representation (e. g. BA 40).

Discussion and conclusions: The findings suggest compensatory processes in ALS patients during visual perception as well as imagery of body movements of everyday life. ALS patients' increased activity in higher order visual areas together with their reduced activity in premotor areas during movement perception might indicate cross-modal compensation to overcome functional loss in motor networks. Progressive impairment of movement execution in ALS might lead to a fading motor memory of these movements. Increased activity in premotor areas and in areas of higher order movement representation might compensate for this in imagery tasks.

P90 SACCADIC EYE MOVEMENTS IN MOTOR NEURON DISEASE AND FRONTOTEMPORAL DEMENTIA

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Keywords: saccadometry, executive dysfunction, frontal dysfunction

Background: Motor neuron disease (MND) and Frontotemporal dementia (FTD) share clinical, pathological and genetic characteristics and saccadic eye movements may be abnormal in MND.

Objective: To characterise saccades in MND compared to FTD and controls using the Linear Approach to Threshold with Ergodic Rate (LATER) model.

Methods: Saccadometry was performed on MND and FTD patients, using a portable saccadometer, and results were compared to matched control subjects. Median reflexive saccadic latency, velocity, and LATER parameters (μ, ς, ςE) were computed, as was performance on an anti-saccade task. Parameters were correlated with cortical atrophy using VBM analysis in FTD patients.

Results: 70 subjects (25 MND; 16 limb-MND, 9 bulbar-MND, 22 FTD and 23 controls) were studied. Median saccadic latency was normal in MND, but prolonged in FTD (MND 201.1 +/- 31.3ms; FTD 229.6 +/- 41.8ms; controls 193.7 +/- 38.7ms, $P < 0.05$). Peak saccadic velocity did not differ in either patient group compared to controls. A measure of decision-making speed (μ) was normal in MND, but reduced in FTD (MND 5.1 +/- 0.9; FTD 4.5 +/- 0.9; controls 5.4 +/- 1.3, $P < 0.05$). ςE was increased in MND and FTD compared to controls, indicating an increased proportion of early saccades in both disease groups (MND 4.0 +/- 2.8; FTD 4.2 +/- 3.0; controls 1.1 +/- 2.4, $P < 0.05$). MND patients performed normally on the antisaccade task, but FTD patients performed poorly (MND 67.9 +/- 12.4%; FTD 56.5 +/- 9.9%; controls 66.7 +/- 13.2%, $P < 0.05$). μ and ςE correlated with atrophy of the left frontal eye field in FTD patients.

Discussion: Both MND and FTD patients had an increased proportion of early saccades, indicated by an increased ςE, which may reflect impaired inhibition of early saccades by higher cortical structures. Decision-making speed, as indicated by μ, was normal in MND, but reduced in FTD. Reduced μ and increased ςE correlated with atrophy of the left frontal eye field.

Conclusion: The only abnormality detected in MND patients was an increased proportion of early saccades, which may reflect subtle frontal lobe dysfunction. In contrast, FTD patients had more widespread abnormalities including an increased proportion of early saccades, reduced median saccadic latency, and poor performance on the anti-saccade task.

P91 ASSESSMENT OF ALS DISEASE PROGRESSION WITH THE SIX-MINUTE WALK TEST

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Keywords: longitudinal assessment, clinical trials, functional impairment

The six-minute walk test (6MWT) has been conventionally used to measure functional exercise capacity in patients with cardiorespiratory disease. Given that functional impairment is an important source of morbidity in amyotrophic lateral sclerosis (ALS), simple measures of exercise capacity need to be validated. The objective of the present study was to investigate the utility of the 6MWT in monitoring disease progression and functional exercise capacity in patients with ALS. 28 ALS patients (20 males; age, 58.3 (SD, 9.4); median disease duration, 24.6 months (interquartile range, 14.2 – 34.4 months)) were recruited from a specialised multi-disciplinary ALS clinic, and evaluated longitudinally for up to 45 weeks. 6MWT, Short Form-36 (SF-36; physical component score) and ALS Functional Rating Scale-revised (ALS FRS-r) were administered. Linear mixed effects models with random intercepts and slopes were used for data analysis. Distance walked over six minutes at baseline was reduced (mean, 65.2% predicted; SD, 17.4% predicted). Distance walked over six minutes declined linearly at a rate of 0.32% predicted per week (S.E., 0.09; $P = 0.0005$). Patients with bulbar-onset disease walked 14.7% predicted (S.E., 0.1% predicted) further than patients with limb-onset disease over the entire study ($P = 0.07$). ALS FRS-r and its gross motor subscore also underwent linear decline at rates of 0.2 units (S.E., 0.03 units; $P < 0.0001$) and 0.1 units (S.E, 0.02 units; $P < 0.00001$) per week respectively. The physical component score of SF-36 did not appear to diminish over time ($P = 0.11$). Rate of decline in 6MWT (% predicted) was correlated with rates of decline in ALS FRS-r (Pearson's $r = 0.57$; $P = 0.002$) and ALS FRS-r (gross motor subscore; Pearson's $r = 0.43$; $P = 0.02$). There was no correlation between 6MWT (% predicted) and the physical component score of SF-36. Given these findings, we propose that the 6MWT may be incorporated as a functional measure of exercise capacity and disease progression in ALS clinical trials.

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THEME 6 THERAPEUTIC STRATEGIES

P92 THE ALSFRS @20: EVOLUTION OF THE ALSFRS-R, HISTORY, CLINIMETRIC PROPERTIES AND FUTURE DIRECTIONS

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Keywords: ALSFRS-R, clinical trials, history

Background: The ALS Functional Rating Scale was developed beginning in 1991 for use in clinical trials of new therapeutic agents for ALS. In the 20 years since its inception, the ALSFRS, now in its revised version as the ALSFRS-R, has become the most frequently used ADL assessment instrument in ALS trials, appearing in more than 175 peer-reviewed publications. The ALSFRS and ALSFRS-R have been applied in other patient management and research settings as well. The ALSFRS-R has become an accepted primary endpoint measure for Phase 3 clinical development.

Objectives: To review the history of the development and use of the ALSFRS and ALSFRS-R.

Methods: This presentation will review the concepts underlying the ALSFRS and ALSFRS-R, the clinimetric properties of the scales, and advances in their application over the last 20 years.

Results: The ALSFRS and ALSFRS-R are reliable and reproducible scales with good clinimetric properties. Their administration is easy to standardize, and it is easy for patients and caregivers to use. They have been shown to be effective in retrospective chart reviews of ALS patients. The ALSFRS and ALFRS-R have been validated for administration over the telephone, and may be administered by computer interface as well. The ALSFRS and ALSFRS-R have been translated into and validated in 8 languages, and have been used in clinical studies world-wide. The scales have been adopted in numerous types of research studies, including online registries, and patient-initiated social networking research projects. The rate of progression of ALSFRS-R from onset of disease has been confirmed to predict survival time in ALS patients. Versions of the ALSFRS have been developed for other neuromuscular diseases (IBMFRS) and for advanced stages of ALS. New methods of analysis have recently been proposed which have the potential to increase the validity of the scale in long-term clinical trials.

Discussion: The ALSFRS, as it has evolved into the ALSFRS-R, continues to be a valuable tool in ALS clinical and drug development research, and has become a template for assessing function in ALS and other neuromuscular diseases. Future evolution and application of the ALSFRS-R will be discussed.

P93 STATISTICAL MODELING TO ILLUSTRATE THE CONTRIBUTION OF AND EFFECTS OF DIFFERENTIAL MORTALITY AND FUNCTIONAL CHANGE ON JOINT RANK TEST OUTCOMES IN ALS

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Keywords: dexpramipexole, joint-rank test, ALSFRS-R

Background: The joint rank test (1,2) is a valid statistical approach to the analysis of functional outcomes adjusted for mortality, for which subjects are ranked on clinical outcomes (change in ALSFRS-R and death). The rankings generally result in deaths being ranked worse than survivors and ranked by time to death; survivors are then ranked by change in ALSFRS-R. Ranked scores are then compared between treatment groups using the Generalized Gehan-Wilcoxon rank test.

In a previously reported (3) Phase 2 study comparing dexpramipexole high dose (300 mg/day) versus low dose (50 mg/day) for 24 weeks in 92 ALS subjects, the high dose group showed a 68% reduction in the hazard of mortality ($p = 0.07$) and a 21% reduction in slope of decline of ALSFRS-R ($p = 0.18$). The slopes result alone probably underestimates the true treatment effect on ALSFRS-R due to the large imbalance in mortality favoring the high dose group. The joint rank test showed an estimated 28% improvement in mean rank ($p < 0.05$) for the high dose group.

Methods: For this abstract, statistical modeling simulations were used to evaluate how the joint rank test behaves under circumstances of concordance and discordance of hypothetical treatment effects on ALSFRS-R and mortality: equal effect on both; strong effect on ALSFRS-R but no effect or negative effect on mortality; and strong effect on mortality but no effect on ALSFRS-R.

Results: Results show that the joint rank test is more appropriate than the slopes analysis of function if there is a strong mortality effect and a moderate effect on function. If effects on mortality and function are similar, there is a modest loss of power with the joint rank test compared to the slopes analysis (due to the non-parametric ranking). However, the joint rank results may be more valid in that they are not dependent on questionable assumptions implicit in para-

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metric models. If there is a strong functional effect and a deleterious mortality effect, the joint rank test will appropriately diminish the level of significance compared to the slopes model. If there is a strong mortality effect and no functional effect, the joint rank test is not as powerful as a survival analysis.

Conclusions: These modeling results illustrate that the joint rank statistic is a reasonable and intuitively appealing primary analysis of function adjusted for mortality. The interpretation of the clinical significance of differences in mean joint rank scores strongly depends on analyses of the component ALSFRS-R and mortality data.

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P94 NOVEL PHASE II TRIAL DESIGN: VARYING COMBINATIONS OF CONTEMPORARY AND HISTORICAL CONTROLS

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Keywords: clinical trials, design, ALSFRS-R

Background: The use of historical controls (HC) in phase II trials is controversial. However, an increasing number of small pharmaceutical companies with limited resources want to use HC for phase IIa trials. To optimize efficiency of trial design, and to mitigate concerns about HC, varying combinations of HC and contemporary controls might be considered.

Objectives: To evaluate varying numbers of contemporary and historical controls for comparison with treated subjects in a phase II trial.

Methods: We investigate a situation where resources limit total sample size to 60 patients. Using simulated data from placebo patients in 5 previous clinical trials, we compared reduction in ALSFRS-R slope over 6 months, that can be detected with 80% power in a variety of phase II designs:

DD1) 30 treated vs. 30 placebo, no HC
DD2) 30 treated vs. 30 placebo + HC
DD3) 50 treated vs. 10 placebo + HC
DD4) 60 treated vs HC

The comparison HC are matched (by entry symptom duration and initial FVC) to those in the trial. For designs with contemporary placebo plus HC (D2 and D3), a favorable outcome is either a significant ($p < 0.05$) difference between the contemporary patient groups (treated vs placebo) OR a significant difference between the treated and the combined contemporary placebo plus HC, after first testing whether contemporary placebo differ from HC.

Results: The minimum slope reduction that can be detected (with 80% power) decreases as we go from D1 to D4. The reduction must be 46% or greater for D1, 37% or greater for D2, 29% or greater for D3 and 27% or greater for D4. For designs D2 and D3, power is 57% (D2) and a 15% (D3) to detect a 30% change in the placebo slope.

Discussion: The most efficient design for detecting a slope reduction is the one where all 60 patients are treated and compared with matching HC (D4). However, this open label design carries the greatest risk of bias, and therefore, the lowest strength of evidence. The hybrid designs, D2 and D3, are less efficient but do provide some check on whether slopes are changing over time, possibly due to improved disease management. The 'conservative' design (D1) that makes no use of HC is the least efficient but may be preferred since its results, if favorable, could be used as one of the two 'registration' trials required for FDA approval.

Conclusion: Varying combinations of contemporary and historical placebos may be a novel way of improving the efficiency of ALS trials. A small contemporary placebo group, as part of a randomized, blinded trial, reduces the risk of bias and raises the strength of evidence compared with an open label trial utilizing HC.

P95 A MODEL TO EXPEDITE PHASE II CLINICAL TRIAL START UP IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: clinical trial research, collaboration, recruitment

Background: Delays in Amyotrophic Lateral Sclerosis (ALS) clinical trial start up are not cost effective and may prevent once eligible subjects from participating in a trial, lowering the potential subject pool for an orphaned disease. Riluzole is currently the sole FDA approved treatment for ALS, a disease for which the average life expectancy is 3-5 years. It is therefore imperative to advance clinical research in ALS free of logistical barriers.

We propose that the employment of a dedicated collaboration model will aid in accelerating clinical trial start-up processes compared with non-collaborative clinical trial submissions.

Objectives: The aim of this pilot study was to demonstrate that a dedicated collaboration with a phase II trial and all participating approving bodies in clinical research can, expedite start up time, improve trial efficiency and increase the projected enrollment period.

Methods: A pilot study was created in collaboration with a phase II trial center, the Contracts Office, and the Institutional Review Board (IRB) to expedite the review of time-sensitive therapeutic trial contracts and protocols. Real time data was collected on a phase II trial using the collaboration model and this was compared to two similar, previously conducted phase II trials that did not utilize this model.

The mean time in days of IRB and contract approval for two ALS phase II clinical trials was compared to the current phase II clinical trial using the collaboration model.

Results: Time from submission to IRB approval was 30 days using the collaboration model as compared to a mean time of 108 days for similar, previously conducted phase II ALS trials. Contractual Trial Agreement (CTA) execution was 28 days, as compared to a mean time of 105 days in previous clinical trials. Clinical research center (CRC) approval was obtained in 67 days. No data was collected in the prior studies on this particular time frame.

Discussion: This study shows that dedicated collaboration with a phase II trial management team and all approving bodies does accelerate start up processes. This acceleration was achieved without compromising trial integrity or forgoing good clinical practice. These results support the proposal to use a similar model for future clinical trials to improve study start-up and trial efficiency and increase potential enrollment.

P96 DATA MONITORING COMMITTEE (DMC) ORGANIZATION IN AN 18-MONTH AMYOTROPHIC LATERAL SCLEROSIS (ALS) SURVIVAL TRIAL: THE EXAMPLE OF A PHASE 3 TRIAL WITH OLESOXIME VS PLACEBO AS ADD-ON TO RILUZOLE

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Keywords: olesoxime, safety, clinical trial

Background: There is an ethical and regulatory obligation to monitor safety in clinical trials. Several recent publications have shown that test treatment could negatively impact function or survival in ALS patients.

Objectives: To describe the organization and functioning of a DMC to ensure patient safety in a phase 3, 18-month ALS survival trial.

Methods: The DMC was composed of a neurologist (chair), a clinical pharmacologist and a biostatistician. Before the trial start the DMC convened with the sponsor to agree on a charter and decide on safety parameters: semi unblinded (treatment A and B) Kaplan-Meir survival curves, ALSFRS, Laboratory values, ECG parameters, adverse events (AE) rated as severe and all SAE reports. Quantitative variables were presented as cumulated distribution function curves per visits in addition to standard descriptive statistics or specific analyses according to guidelines (e.g., QTc). The safety report was prepared by an independent statistician using an automated process, extraction from the e-CRF database, analysis with R and production of the document with LaTeX. A blinded version of the safety report was provided to the sponsor and the trial steering committee. Two formal tests of worsening of survival were scheduled to be conducted when 50 and 100 events had occurred (total sample size N = 470, 150 events expected). Survival simulations were conducted to assess if trial prolongation could be required to record the expected number of events to ensure power of the study to detect treatment differences. The DMC holds closed meetings every 3 months and issues a recommendation to the trial steering committee to either continue as planned or advises otherwise.

Results: This DMC organization provides adequate way of ensuring patient safety. The interpretation of severe AEs was difficult due to the lack of online MedDRA coding. Survival simulations were conducted because the number of events appeared initially to be lower than expected with an exponential model. A Weibull model fits the data better and with no need for a trial prolongation.

Conclusion: This process provides a DMC with online and adequate safety information to allow patient protection. Training of neurologists in roles and responsibilities of being on a DMC should be encouraged.

P97 SUCCESSFULLY TARGETING ALS/MND THERAPIES: THE DIAPHRAGM PACING EXAMPLE OF UTILIZATION OF THE FDA HUMANITARIAN PATHWAY FOR EXPEDIENT AND COST EFFECTIVE ACCESS TO NEW THERAPIES IN AN ORPHAN DISEASE

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Keywords: diaphragm, humanitarian, regulatory

Background: Diaphragm Pacing (DP) has been developed as a treatment for respiratory insufficiency in persons with ALS based on the successful use in high level spinal cord injury.(1,2, 3) The U.S. FDA Humanitarian Use Device (HUD) designation provides a regulatory pathway to bring devices to market in orphan diseases. Once designated as a HUD, evidence may be presented, to FDA, of safety and probable benefit for Humanitarian Device Exemption (HDE) market approval. DP has successfully navigated these pathways for ALS.

Objectives: Review the entire regulatory pathway to bring a novel new therapy for ALS/MND

Methods: Retrospective analysis of all of the regulatory pathways to commercialize DP.

Results: DP initial success in pure upper motor neuron (UMN) of SCI led to the initial FDA investigator initiated investigational device exemption (IDE) and IRB applications which began in 2003 with approval in 2004 for the pilot trial with first surgical implantation in 2005. Success of the pilot trial led to the FDA approved multi-center pivotal trial with first implantations in 2007. Prior to start of the trial the IDE was transferred to a commercial entity (Synapse Biomedical) and funding for the trial was raised through venture capital. The final one year patient follow-up occurred at the end of 2009 which allowed statistical analysis. There was a delay in obtaining HUD designation which needed a medically plausible subset of less than 4,000 US patients a year until an agreement was reached in 2010. HDE application was approved for probable benefit in ALS patients in 2011.

Discussion: The HDE pathway is rarely used in the U.S. compared to other means. In 2009 there were approximately 3,000 510(k) approvals, 15 original Pre-Market Approvals (PMA)'s and only 1 HDE approved. With the average total cost, from concept to approval, of a higher-risk PMA device of US$94 million; the return on investment for a company to develop devices in orphan diseases is severely limited.

Conclusions: DP is the first humanitarian device to be approved for ALS; it may also be the first device with an explicit indication for ALS. DP has been shown, using the HDE pathway, to be safe and of benefit in the treatment of respiratory insufficiency in ALS. The HDE pathway is a cost effective means to bring therapies to market for ALS patients. The success of DP will allow more commercial entities to invest in developing new therapies to improve the quality of life in patients with ALS/MND.

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P98 PRELIMINARY EVALUATION OF THE IMPACT OF CONCOMITANT RILUZOLE ON DEXPRAMIPEXOLE TREATMENT EFFECTS IN ALS

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Keywords: dexpramipexole, ALSFRS-R, riluzole

Background and objectives: Dexpramipexole, a drug that may ameliorate mitochondrial dysfunction, is being developed to treat ALS. Riluzole is frequently used for ALS patients since its approval in 1996. The objective of this study is to report whether treatment with concomitant riluzole (non-randomized) modulates the treatment effects of dexpramipexole in a previously reported (1) small Phase 2 study.

Methods: The second part of this two-part, randomized, multicenter, double-blind, placebo-controlled study, was selected for analysis because power was increased with the greater per group sample size relative to part one. A total of 92 ALS patients were randomized to receive 50 or 300 mg/day dexpramipexole for 24 weeks. A total of 54 subjects were receiving concomitant riluzole (25 of 48 subjects in the 50 mg/day group; 29 of 44 subjects in the 300 mg/day group). Clinical outcomes were, in part, measured by the ALSFRS-R and survival. For this abstract, dexpramipexole treatment effects on functional decline and survival were examined in the subgroups of subjects receiving and not receiving concomitant riluzole.

Results: Subjects who received 300 mg dexpramipexole showed a trend towards a better ALSFRS-R slope (1.02) than subjects who received 50 mg (-1.28, $p = 0.18$), an improvement of 21%. The treatment effect of dexpramipexole on ALSFRS-R slope was similar in subjects receiving riluzole (-1.09 for 300 mg, -1.38 for 50 mg) and in subjects not receiving riluzole (-0.89 for 300 mg, -1.19 for 50 mg). There was no evidence of an interaction between dexpramipexole and riluzole on slope of ALSFRS-R ($p = 0.98$).

The 24-week mortality rate was lower in the dexpramipexole 300 mg group than in the 50 mg group, 7.2% vs 19.9%, which corresponds to a reduction in hazard rate of 68%. The effect of dexpramipexole on mortality was independent of concomitant riluzole use. In subjects taking concomitant riluzole, the Kaplan-Meier estimates (SE) were 17.2% (7.9%) in the 50 mg group and 7.1% (4.9%) in the 300 mg group. In subjects not taking concomitant riluzole, the estimates (SE) were 22.7% (8.9%) in the 50 mg group and 7.1% (6.9%) in the 300 mg group. The numbers of subjects in each cell (+/- dexpramipexole and +/- riluzole) were too small to warrant statistical evaluation of interaction effects.

Discussion and conclusions: In this small Phase 2 study, results suggest that concomitant riluzole treatment neither augments nor diminishes the treatment effect of dexpramipexole on survival or functional decline in ALS. It will be useful to confirm this observation in the definitive Phase 3 studies of dexpramipexole in ALS.

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P99 FIRST IN HUMAN PHASE 1 TRIAL OF NEURAL PROGENITOR CELLS (NEURALSTEM) IN ALS: RESULTS IN THE FIRST 12 PATIENTS

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Keywords: stem cells, human trial, spinal cord injections

We have completed 12 patient surgeries in this Phase 1 trial. Patients with ALS have been injected with fetal-derived neural stem cells into the lumbar spinal cord. The design is one of "escalating risk", where each group of patients are progressively less impaired. The first 6 patients were non-ambulatory, 3 of whom were supported by mechanical ventilation. The first 3 patients received 5 unilateral injections at L2-L4, and the next 3 received 5 injections bilaterally (total 10 injections) at the same levels. Patients 7-12 were ambulatory and had vital capacities > 60 % predicted. Patients 7-9 received 5 unilateral lumbar spinal cord injections, and patients 10-12 received bilateral lumbar injections. Each injection had a volume of 10 µl and a cell concentration of 10,000 cells/µl, so patients received either 500,000 or 1 million cells through 5 or 10 injections, respectively. The injection apparatus was fixed to the patient's spine, and so was able to move along with patient movement ("floating cannula") and avoid any lateral shear during the operation.

As of early May, 2011, there has been one death, unrelated to either ALS or the clinical trial. All patients tolerated the surgical procedure with minimal perioperative or postoperative problems. There have been no adverse events attributable to the cellular injections. Patients are immunosuppressed with a combination of tacrolimus and mycophenolate, which resulted in gastrointestinal distress in some patients.

The trial is continuing on schedule. We are using clinical evaluation, strength testing, and electrical impedence myography to monitor progression of disease. Following FDA review and approval of safety data from the first 12 patients we will move to injections into the cervical spinal cord. The conference presentation will provide up to date information on our progress to date.

P100 METALLOTHIONEIN DISPLAYS NEUROPROTECTIVE EFFECTS ON DORSAL ROOT GANGLION NEURONS AND EXTENDS SURVIVAL IN SOD1(G93A) MICE

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Keywords: metallothionein, emtin

Background: The SOD1(G93A) transgenic mouse develops limb paralysis and spinal cord pathology that is similar to mutant SOD1-linked cases of ALS. Several studies have reported the upregulation of the neuroprotective metal-binding protein metallothionein (MT) in SOD1(G93A) mice and in ALS spinal cord. Additionally, SOD1(G93A) mice deficient in MT have a hastened disease endpoint.

Objective: In this study, we have used tissue culture to investigate whether MT is able to stimulate repair of injured axons. We have also investigated whether the neuroprotective effect of MT can be used advantageously to produce a better outcome for ALS.

Methods: In the *in vitro* experiments, explant or dissociated cultures of dorsal root ganglion (DRG) neurons from embryonic day 15 Sprague Dawley rats were maintained in Neurobasal medium containing 10% B-27 supplement. In some cases, dissociated DRG neurons were maintained in Campenot chambers, which allowed separation of cell bodies and processes, allowing precise evaluation of the effect of MT upon regeneration of injured DRG neurons. In the in vivo experiments MT was administered to SOD1(G93A) mice at 1mg/kg body weight twice weekly, via either unilateral or bilateral intramuscular injection. Mice received injections on one of two administration schedules: either commencing at 10 weeks of age and continuing until endpoint (during the symptomatic period); or for a period of 10 weeks commencing at 6 weeks of age (during the pre-symptomatic period). Motor impairment was assessed by hindlimb grip-strength, stride pattern and weight. Additionally, peptide analogues of MT ('emtins') were administered subcutaneously at 10mg/kg three times weekly from 12 weeks of age until endpoint.

Results: The results showed that MT can act neuroprotectively and stimulate nerve sprouting and regeneration of injured DRG neurons. The actions of MT involved interaction with the low-density lipoprotein (LRP) family of receptors, since a competitive LRP ligand (receptor associated protein) and the MAPK inhibitor PD98059 both blocked the actions of MT. Symptomatic intramuscular injection of MT resulted in an approximate 5% improvement in survival and a notable preservation in motor function. However, pre-symptomatic intramuscular injection of MT resulted in preservation of motor function but did not significantly increase survival. This indicates that MT may be acting directly on the peripheral nerves, or indirectly on the muscle, when injected intramuscularly throughout the symptomatic period. Symptomatic emtin peptide administration resulted in a similar improvement in survival, but did not appear to maintain motor function.

Discussion and conclusions: So far the results suggest that exogenous MT has a neuroprotective effect on injured nerves, possibly acting through LRP receptors. Administration of MT or emtin peptides may have a therapeutic benefit in SOD1(G93A) mice, which may be related to the neuroprotective effect of MT on peripheral nerves.

P101 THERAPEUTIC EFFECTS OF A FIRST-IN-CLASS THYROTROPIN-RELEASING HORMONE (TRH)-BASED LEAD COMPOUND IN THE G93A SOD1 TRANSGENIC MOUSE

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Keywords: neurotherapeutic, TRH-based therapeutics, treatment

Background: Drugs that target multiple neuropathological mechanisms rather than a single mechanism may offer distinct therapeutic advantages in ALS treatment (1). Thyrotropin-releasing hormone (TRH) is a naturally-occurring neuropeptide with multifactorial neurotherapeutic effects relevant to ALS (2–4). Harnessing TRH's clinical potential has proved to be difficult because of its short half-life and potential neuroendocrine side effects (1–3). The ground-breaking first-in-class TRH-based lead compound (LC) we have developed overcomes these limitations and the weaknesses of existing TRH analogs. LC, a new chemical entity (patent application submitted), provides an innovative means to unlock the recognized medical potential of TRH-based neurotherapeutics in ALS treatment.

Objectives: To determine the ability of LC to provide therapeutic benefit in the G93A SOD1 transgenic mouse model of ALS.

Methods: Effects of LC in male G93A SOD1 transgenic familial ALS mice (high copy number; B6SJLTg (SOD1-G93A) 1Gur/J; The Jackson Laboratory, Bar Harbor, ME, USA) were investigated in a randomized, blinded, gender-weight- and age-matched study performed according to guidelines for preclinical animal research in ALS/MND. Treatment with LC (2 mg/kg i.p. 5 days/week) or vehicle was initiated in 2 randomly assigned groups ($n = 13$ per group) 6 weeks after birth and continued until end stage (death or when the mouse is unable to right itself within 30 seconds). Readouts were rotarod performance, body weight and survival.

Results: Mean rotarod performance was essentially constant until disease onset, which was observed to occur at 100 days, as indicated by a sudden 11% reduction in motor performance in both vehicle- and LC-treated mice. Following onset, motor function and mean body weight rapidly deteriorated in vehicle-treated animals. LC treatment consistently significantly improved rotarod performance and reduced weight loss after disease onset (two-way repeated measures ANOVA; $n = 13$, $p < 0.01$, Newman Keuls post-hoc test, for both readouts). Rotorod performance was increased by 21% at first measurement following onset (day 105) and increased to 109% on last day of measurement before first death (day 118); body weight was increased by an average of 6% over the same period. Median survival for LC-treated group was 132 days compared to 126 days for vehicle-treated animals.

Discussion and conclusions: Pre-symptomatic treatment with LC provides significant positive effects in two important measures of therapeutic benefit, namely motor function and weight. Data indicate for the first time that LC modifies the disease process in this ALS model and has potential to provide an innovative therapy for ALS patients.

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P102 ASC-J9 ENHANCES THE MUTANT TDP-43 DEGRADATION BY AUTOPHAGY

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Background: Mutations in the Cu,Zn-superoxide dismutase (SOD1) gene cause a familial form of amyotrophic lateral sclerosis (ALS) through an unknown gain-of-function mechanism. Mutant SOD1 aggregation may be the toxic property. Recently, 43-kDa TAR DNA-binding protein (TDP-43) was identified as the major disease protein found in ALS and frontal temporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U). Mutant TDP-43 induced neuron damage, including apoptosis, oxidative stress, autophagy dysfunction and neurite outgrowth inhibition. P62 (also referred to as SQSTM1) is an adaptor scaffold protein, which binds both to polyubiquitinated proteins in aggregates and to LC3. P62 is one of the components of the ubiquitin-containing inclusions in several human neurodegenerative diseases. It plays important roles in forming inclusions and may be associated with the protection of neurons from degenerative processes.

Objective: To test natural products and their derivatives for selective degradation of misfolded proteins and protecting cells from the toxic effects of misfolded or mutated proteins.

Methods: To examine the solubility of TDP-43, sequential extractions were performed using RIPA buffer and urea buffer. P62 siRNA and mutant TDP-43 plasmids were transfected by using Lipofectamine following the manufacturer's instruction. The immuno-precipitation was carried out to detect the interaction of p62 and TDP-43. Western blot and Confocal microscopy analysis were used to test the expresson of TDP-43. TBARS in the cells and LDH content in the medium were tested.

Results: We found that 5-hydroxy-1,7-bis(3,4-dimethoxyphenyl)-1,4,6-heptatrien-3-one (ASC-J9) treatment up-regulated nrf2-dependent expression of p62, activated autophagy. It promoted p62-dependent degradation of mutant SOD1, mutant full length TDP-43 and the ~25, 35 kDa C-terminal fragment of TDP-43. But it had little effect on WT-TDP-43 degradation. The depletion of p62 decreased the recruitment of LC3 to mutant TDP-43, partly inhibited the degradation of mutant SOD1 and TDP-43. ASC-J9 also restored redistribution of endogenous TDP-43 from the cytoplasm to nucleus, activated Nrf2 and Nrf2 target genes and decreased oxidative damage. Interestingly, ASC-J9 promoted neurite outgrowth significantly.

Discussion: We found that autophagy contributes to the clearance of aberrant TDP-43 proteins. One main function of autophagy is to selectively eliminate toxic protein or damaged organelles. P62 can differentiate between WT and mutant toxic protein through levels of ubiquitination and increased the recruitment of LC3 to mutant protein. Enhancing the function of selective autophagy and increaseing the expression of p62 with drugs may be a tractable therapeutic strategy for associated diseases. ASC-J9 ameliorated the mutant protein-induced toxic effects by activating selective autophagy.

Conclusion: ASC-J9 may be a potential compound for neurodegenerative diseases.

P103 PROTECTIVE EFFECT OF DIMETHOXY CURCUMIN ON MITOCHONDRIAL DYSFUNCTION IN MUTATED HUMAN TDP-43 TRANSFECTED NSC34 CELL LINE

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Keywords: dimethoxy curcumin, mitochondrial dysfunction, TDP-43

Background: Recently, one of the major disease proteins found in the pathological inclusions of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U) was identified as the TAR-DNA-binding protein of 43 kDa (TDP-43). Mitochondrial damage in TDP-43 linked animal and cell models have been reported. So we further studied the mitochandial function in our cell models.

Objectives: To further elucidate mitochondrial dysfunction in TDP-43 cell models, as well as to identify therapeutic approaches that target mitochondrial dysfunction and its consequences.

Methods: Mitochondrial morphology was tested by electron microscopy, mitochondrial complex I, and activity by spectrophotometric assays, mitochondrial member potential by flow cytometry and mitochondrial uncouple protein 2 expression level by Western Blot in four stably transfected cell lines. Then we observed the protective effect of dimethoxy curcumin (DMC) on mutated human TDP-43 cell line.

Result: Mutated human TDP-43 caused mitochondrial morphologic abnormality including mitochondrial swelling and disturbance of mitochondrial cristae, decreased complex activities, depolarized mitochondrial membrane potentials, and increased expression of mitochondrial uncoupling protein 2 (UCP2) in the NSC-34 cells. Meanwhile we found that the abnormal changes in mitochondria would be restored by DMC in mutated TDP-43 transfected cell lines.

Discussion: Studies on pathogenesis of TDP-43 are promising. Both gain and loss of TDP-43 functions are potential disease mechanisms. Mitochondrial dysfunction involves pathogenesis in ALS patients and in a variety of experimental models of SOD1-linked fALS. Our study showed that mitochondrial dysfunction exists in this TDP-43 cell model. Notably, DMC can ameliorate mitochondrial function in mutated TDP-43 transfected cell lines.

Conclusions: Mitochondrial dysfunction might be a common pathogenic mechanism in SOD1 related or TDP-43 related ALS. DMC could be a potential therapy approach in neurodegenerative diseases linked with mutated TDP-43.

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THEME 7 *IN VIVO* EXPERIMENTAL MODELS

P104 PRECLINICAL TRIAL OF A GSK3 AND PDE7 DOUBLE INHIBITOR IN SOD1G93A MICE: A GENDER SPECIFIC EFFECT

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Keywords: preclinical assay, GSK-3, PDE-7

Background: Glycogen synthase kinase (GSK)-3 has recently been implicated in the pathogenesis of neurodegenerative diseases. This enzyme is currently used as a neuroprotector agent and it modulates the apoptotic pathway because the inhibition of GSK-3 stops the intrinsic apoptosis (1).

cAMP and cGMP are inactivated by cyclic nucleotide phosphodiesterases (PDE), then these enzymes modulate cyclic nucleotide-mediated signal pathway like neuroinflammatory responses (2,3). From the different phosphodiesterases expressed, PDE-7 is expressed in T-cells, brain and skeletal muscles.

The GSK-3 and PDE7 inhibition could prevent motor neuron apoptosis, increase levels of cAMP in muscle of ALS patients and repress neuroinflammatory symptoms.

VP1.15 was originally synthesized as an inhibitor of GSK-3 but also presents inhibitory capacity for PDE7 (with IC = 2 microM and IC = 1.11 microM respectively).

Objectives: The aim of this project was to prove VP1.15 in the transgenic hSOD1 mutant mice developing a preclinical assay.

Methods: A total of 30 transgenic female mice and 22 transgenic male mice were recruited (all of them tested for the human transgene). In a previous study in wild type mice we determined that a dose of 4 mg/kg was not toxic.

The trial began at the time of onset of symptoms (87.5 ± 2.3 days of life) that were analyzed by taking the weight three times a week, by visual assessment of motor neuron impairment and by testing grid once a week. The previous selected dose was administrated by intraperitoneal injection during the following 35 days.

Results: Medium survival: Males - untreated 127/treated 138.5 days; Females - untreated 142/treated 144 days. Kaplan-Meier survival analysis gives the following values of p all animals: $p = 0.7403$ (females); $p = 0.0774$ (males); values obtained from littermates: $p = 0.8027$ (females); $p = 0.0667$ (males).

There are not obvious differences in the evolution of weight in treated and untreated males. However, treated females have less weight loss compared to untreated.

No differences were found when evaluating the neuromuscular deterioration between treated and untreated females. By contrast, neuromotor involution of untreated males is faster than treated males. Treated males exhibit a progressive loss of neuromuscular skills to rates similar to females. Similar findings were obtained in grill test.

Discussion and conclusions: Males respond to the treatment improving their survival about 10 days and no effect was apparently observed in females. The big differences between sexes could be related with the inhibition of VP1.15 that simulates the protective effect of estrogens, which was proven in this model (4). Subsequent studies are necessary to test the positive effect of VP1.15 in an ALS model in which senescent females develop the disease.

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P105 RESCUE OF ADULT MOTONEURONES BY MECHANO-GROWTH FACTOR (MGF), LIVER-TYPE INSULIN-LIKE GROWTH FACTOR -1 (IGF-1EA) AND GLIAL CELL DERIVED NEUROTROPHIC FACTOR (GDNF) DELIVERED AT THE TIME OF NERVE INJURY

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Keywords: neurotrophic factors, adult, motoneurone

While much is known of the neurotrophic rescue of immature motoneurones, little is known about their rescue in the adult animal. We previously found that intramuscular transfer of the gene for a muscle-derived isoform of insulin-like growth factor-1 (MGF) one week before facial nerve avulsion rescued 80% of adult rat motoneurones when 80% would have died,

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and that it was twice as effective than the commonly-used liver-type isoform of IGF-1 (1). In this study we have examined the neuroprotective potential of MGF and IGF-1 peptides delivered at the time of facial nerve injury in adult rats and compared this with a known potent rescue factor for immature rat facial motoneurones, GDNF.

The facial nerve of groups of 4-8 anaesthetised 3m Sprague-Dawley rats was avulsed and 10µl of 1µg/µl of either MGF, liver-type IGF-1 or GDNF injected into the stylomastoid foramen. Control animals received 10µl of saline after avulsion, avulsion only or were non-operated. After 7 days, numbers of motoneurones were determined stereologically. Two additional groups of rats were also examined 14d and 28d after MGF injection.

Seven days following avulsion only, or avulsion + saline, 32% of adult motoneurones were lost. Injection of MGF, liver-type IGF-1 or GDNF immediately after avulsion reduced the motoneuronal loss to 19-21% ($p < 0.05$ vs. controls). Motoneuronal loss increased to approximately 50% 14-28d after MGF injection, while 80% were lost 28d following avulsion alone.

We show that MGF peptide delivered at the time of nerve injury has a similar neuroprotective effect to IGF-1Ea for mature motoneurones at 7 days. Rather than conferring absolute rescue, however, a single dose of MGF peptide reduces the rate of motoneuronal loss up to 28 days. We do not know if the neuroprotective for IGF-1Ec is maintained beyond 7 days, although intramuscular gene transfer of IGF1Ea rescues approximately 50% of adult rat facial motoneurones at 1 month (1). In contrast to intramuscular gene-transfer, injection of adenoviral vectors carrying genes for IGF-1 into the styolomastoid foramen at the time of adult rat facial nerve avulsion is not associated with motoneuronal rescue at 1 month (2).

Taken together, these results indicate that the mode of neurotrophic factor delivery and the post-operative time period examined in experimental models of motoneuronal degeneration can significantly affect the extent of neurotrophic rescue observed.

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P106 MELITTIN EFFECTS IN AN ANIMAL MODEL OF ALS

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Keywords: hSOD1G93A, melittin

Amyotrophic lateral sclerosis (ALS) is a paralyzing disorder characterized by the progressive degeneration and death of motor neurons and occurs both as a sporadic and familial disease. Mutant SOD1 (mtSOD1) in motor neurons induces vulnerability to the disease through protein misfolding, mitochondrial dysfunction, oxidative damage, cytoskeletal abnormalities, defective axonal transport- and growth factor signaling, excitotoxicity, and neuro-inflammation.

Melittin is a 26 amino acid protein and is one of the components of bee venom which is used in traditional Chinese medicine to inhibit cancer cell proliferation and is known to have anti-inflammatory and anti-arthritic effects.

The purpose of the present study was to determine if melittin could suppress motor neuron loss and protein misfolding in the hSOD1^{G93A} mouse, which is commonly used as a model for inherited ALS. Meltittin was injected at the 'ZuSanLi' (ST36) acupuncture point in the hSOD1^{G93A} animal model. Melittin-treated animals showed a decrease in the number of microglia and in the expression level of phospho-p38 in the spinal cord and brainstem. Interestingly, melittin treatment in symptomatic ALS animals improved motor function and reduced the level of neuron death in the spinal cord when compared to the control group. Furthermore, we found an increased of a-synuclein modifications, such as phosphorylation or nitration, in both the brainstem and spinal cord in hSOD1^{G93A} mice. However, melittin treatment reduced a-synuclein misfolding and restored the proteasomal activity in the brainstem and spinal cord of symptomatic hSOD1^{G93A} transgenic mice.

Our research suggests a potential functional link between melittin and the inhibition of neuroinflammation in an ALS animal model.

P107 OLESOXIME (TRO19622) DELAYS MUSCLE DENERVATION, ASTROGLIOSIS, MICROGLIAL ACTIVATION AND MOTONEURON DEATH IN THE MURINE ALS MODEL SOD1G93A

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Keywords: olesoxime, therapy, SOD1G93A

Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative pathology and riluzole, the only approved treatment, delays death by only a few months. The disease is mimicked to a striking degree in transgenic mice carrying human ALS-linked SOD1 gene mutations. These mice have therefore been extensively used to test potential therapeutic molecules. Olesoxime (TRO19622), a novel neuroprotective and reparative compound identified in a high-throughput screen based on motoneuron survival, delays disease onset and improves survival in mutant SOD1^{G93A} mice, a model for ALS (1).

Objectives: The present study further analyses the cellular basis for the protection provided by olesoxime at the neuromuscular junctions (NMJ) and the spinal cord.

Methods: Studies were carried out at two disease stages, 60 days, presymptomatic and 100 days, symptomatic. Cohorts of wildtype and SOD1^{G93A} mice were randomized to receive placebo or olesoxime-charged food pellets from day 21 onward. Body weight was recorded weekly. At 60 days, some mice were sacrificed to measure olesoxime levels in blood, brain and spinal cord. The others were anesthetized and their gastrocnemius muscles dissected out. After post-fixation, cryostat sections were analysed for NMJ integrity by double staining for a-bungarotoxin and neurofilament. At day 100, the same procedure as at day 60 was applied with in addition fixative perfusion after taking out the gastrocnemius and dissection of the diaphragm as well as of the spinal cord. Whole-mount immunostaining was used to study the diaphragm innervation and cryostat sections of the spinal cord were immunostained for VAChT to visualize motoneurons, GFAP for astrocytes and Iba-1 for microglial cells.

Results: Analysis at 60 days showed that olesoxime greatly protects the gastrocnemius muscle from denervation, reducing denervation from 60 to 30% compared to SOD1^{G93A} mice fed with placebo food pellets (n = 5 mice/group). At the symptomatic stage, a preliminary analysis suggests that only a few NMJs were still preserved by olesoxime treatment both in gastrocnemius muscle and in the diaphragm. In the spinal cord, olesoxime strongly reduced astrogliosis and microglial activation and moderately prevented motoneuron loss (n = 3 mice/group). In addition, time of onset defined as the time mice started to loose weight was slightly delayed by olesoxime. Additional mice are currently being evaluated at 100 days to further confirm these results.

Discussion and conclusion: Though still incomplete, these studies suggest that olesoxime exerts its protective effect on multiple cell types implicated in the disease process in SOD1 mice, slowing down muscle denervation, astrogliosis, microglial activation and motoneuron death. A Phase 3 clinical study in ALS patients will determine whether olesoxime could be beneficial for the treatment of ALS.

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P108 ABERRANT NEUREGULIN1 SIGNALING IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: neuregulin, microglia, motor neuron

Background: Neuregulin1 (NRG1) is a neuronally-expressed factor that supports axoglial and neuromuscular development through a number of alternatively spliced isoforms, but has not been studied in the pathogenesis and progression of amyotrophic lateral sclerosis (ALS). NRG1 is an important molecule for axoglial communication in the central nervous system and may be a new potential therapeutic target for patients with ALS.

Objectives: Here, we analyzed the relationship of NRG1 isoform expression with glial cell activation and motor neuron loss in the spinal cords of ALS patients as well as during disease progression in the ALS – superoxide dismutase 1 (SOD1) mouse model.

Methods: Pathological changes were measured for motor neuron number, demyelination, gliosis and microglial activation in both ALS patients and ALS-SOD1 (G93A) mice. Gene expression and protein levels were determined by quantitative PCR for a number of genes implicated in the disease process as well as the NRG1 gene isoforms that have important roles in axoglial interaction.

Results: Significant pathological changes that could be mediated by NRG1 signaling, including microgliosis, astrocytosis and motor neuron loss, were observed in the ventral horn spinal cord in both ALS patients and increased in SOD1 mice, as a function of disease progression. Whereas type III (membrane-bound) NRG1 expression was reduced in parallel with motor neuron loss, type I (secreted) NRG1 increased and was associated with glial activation. Consistently, excessive NRG1 receptor activation was observed on activated microglia in both ALS patients and in SOD1 mice. This activation was observed prior to upregulation of NRG1 gene expression, at the time of disease onset.

Discussion and conclusions: While the downregulation of membrane-bound type III NRG1 forms may be a marker of motor neuron loss, increased signaling by secreted type I NRG1 forms could contribute to disease pathogenesis through glial cell activation and could therefore represent a novel therapeutic target against disease progression in ALS.

P109 IMPAIRED NEUROPROTECTIVE RESPONSE OF MICROGLIA TO ACUTE NEURON INJURY AT THE DISEASE PROGRESSION STAGE IN ALS MODEL

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Keywords: mSOD1, microglia, axotomy

Background: Dominant mutations in superoxide dismutase (mSOD1) cause a familial form of amyotrophic lateral sclerosis (ALS). Accumulating evidence indicates the occurrence of non-cell-autonomous motor neuron death in ALS models based on mSOD1. Activated microglia are seen in both human ALS and animal ALS models. Selective lowering of mSOD1 levels in microglia prolongs survival of mSOD1-Tg mice. Microglia play a significant role in disease progression; however, whether microglia are neurotoxic or neuroprotective remains undetermined. Motor nerve axotomy induces acute motor neuron damage and subsequent microglial responses.

Objectives: We aimed to elucidate whether microglial function is neurotoxic or neuroprotective to motor neurons in an ALS model, by studying temporal changes in microglial responses to acute motor neuron injury in mSOD1-Tg mice.

Methods: We performed unilateral hypoglossal nerve axotomy in mSOD1 (G93A) Tg mice in the presymptomatic stage (8 weeks of age) and the disease progression stage (17 weeks) and their non-transgenic littermates (NTG). On days 3 and 21 after axotomy the medulla, including the hypoglossal nucleus, was removed and sectioned, Nissl-stained for neuronal counting and immunostained with Iba1 for microglial counting. The numbers of neurons and microglia were counted. We also immunohistochemically examined the expression of neurotrophic factors including GDNF and IGF-1.

Results: Hypoglossal nerve axotomy resulted in recruitment of microglia and their encircling of hypoglossal motor neurons at three days after axotomy, which was followed by neuronal loss at 21 days after axotomy. There was no significant difference in the survival rates of motor neurons between mSOD1-Tg and NTG mice at three weeks after axotomy during the presymptomatic stage. By contrast, the survival rate of neurons in mSOD1-Tg mice was significantly lower than that in NTG mice after axotomy during the disease progression stage. There was no difference in the numbers of neurons between axotomized and non-axotomized sides at three days after axotomy. The number of microglia was

significantly lower in mSOD1-Tg mice than in NTG mice during the disease progression stage, but no difference was seen during the presymptomatic stage. Activated microglia of mSOD1-Tg and NTG mice expressed neurotrophic factors including GDNF and IGF-1 during both the presymptomatic and disease progression stages.

Discussion and conclusion: In this study, we found that mSOD1 attenuates microglial responses to acute motor nerve injury during the disease progression stage, but not during the presymptomatic stage. Our findings indicate that reactive microglia are potentially neuroprotective after axotomy. Microglia are thought to heterogeneously include neurotoxic M1 and neuroprotective M2 phenotypes. Impaired neuroprotection from microglia may partly underlie ALS progression. The activation of neuroprotective microglia and normalization of cross talk between neurons and microglia during the disease progression stage may be a target of novel therapies for ALS.

P110 EVIDENCE FOR INVOLVEMENT OF THE INNATE IMMUNE TOLL-LIKE RECEPTOR SYSTEM IN THE SOD1G93A TRANSGENIC MOUSE MODEL OF MOTOR NEURON DISEASE

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Keywords: Toll-like receptors, SOD1G93A mice, neuroinflammation

Background: There is increasing evidence for a role of innate immune system components, such as the complement cascade and Toll-like receptors (TLRs), in the pathogenesis of motor neuron disease. Upregulation of numerous complement factors has previously been shown in mouse models of motor neuron disease. There is also evidence for a role of the TLRs in motor neuron disease; mRNA for several TLRs was shown in post-mortem brain tissue from human motor neuron disease patients, as well as a preliminary report of TLR expression in the mouse SOD1^{G93A} transgenic model of motor neuron disease.

Objectives: The present study aimed to fully characterise the expression of several TLRs (TLR 2, 3, 4 and 7), and one of their endogenous ligands (high mobility group box 1; HMGB1) in SOD1^{G93A} mice.

Methods: Expression of the above factors was compared across various pre-defined stages of the disease (presymptomatic, P28; onset stage, P65; mid-onset stage, P115; and end-stage, P175) in the lumbar spinal cord of C57BL6/J SOD1^{G93A} transgenic mice (n = 9), and their wild-type littermates (n = 9) using quantitative polymerase chain reaction (qPCR). Localisation of TLR2 and TLR4 in the lumbar spinal cord of end stage SOD1^{G93A} mice was also investigated using immunohistochemistry.

Results: mRNA expression of TLR 2, 3, 4 and 7 and HMGB1 was significantly increased in SOD1^{G93A} mice compared to wild-type mice at the end-stage of the disease. TLR3 and HMGB1 had a two-fold increase, TLR4 a five-fold increase, TLR2 a seven-fold increase, and TLR7 an eight-fold increase in mRNA expression in SOD1^{G93A} mice compared to their wild-type littermates. TLR 3, 4, 7 and HMGB1 had no apparent change at mid-onset stage. By contrast, TLR2 expression doubled at the mid-onset stage in SOD1^{G93A} mice. Immunohistochemistry demonstrated that TLR4 was mainly localised to astrocytes, and TLR2 to microglia, at end stage of disease.

Discussion and conclusions: These results suggest that the secondary effect of activating TLR pathways may contribute to the inflammatory progression in this SOD1^{G93A} transgenic mouse model of motor neuron disease. Thus, further understanding the role of TLR activation in neuroinflammation is warranted, and may provide a potential novel therapeutic approach for treating motor neuron disease.

P111 UPREGULATION OF COMPLEMENT COMPONENTS IN MOTOR NEURON DISEASE

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Keywords: neuroinflammation, complement system, CD88

Background: There is increasing evidence that neuroinflammation drives progression of many neurodegenerative diseases. The complement system, which is part of innate immune system, has recently been implicated in the pathogenesis of motor neuron disease (MND). Our previous studies in the hSOD1^{G93A} rat model of MND demonstrated that excessive complement activation in the lumbar spinal cord contributed to motor neuron death. We have shown that hSOD1^{G93A} rats treated with the selective C5a receptor (CD88) antagonist PMX205 had reduced gliosis and improvements in behavioral deficits, consistent with reduced neuropathology.

Objectives: The current study aimed to determine the expression and localization of C5a receptors, CD88 and C5L2 at both the mRNA and protein levels in the hSOD1^{G93A} mouse model of MND and in MND patients.

Methods: Lumbar spinal cord from high-copy number C57BL/6J hSOD1^{G93A} mice and their wild-type (WT) littermates were obtained at 4 different ages during disease progression, and expression and localization of CD88 and C5L2 were examined using qPCR, *in situ* hybridization, Western blotting and immuno-histochemistry. Circulating levels of C5a were also examined using an ELISA. Expression of CD88 in the motor cortex of MND patients were investigated using immunohistochemistry.

Results: We found consistent upregulation of plasma C5a levels which correlated with disease progression, and also upregulation of CD88 and C5L2 mRNA and protein levels in the lumbar spinal cord during disease progression. Immuno-localization showed that CD88 is expressed predominantly on the microglia surrounding the regions of motor neuron death. By contrast, the alternative C5a receptor C5L2 is expressed predominantly on astrocytes. CD88 was also upregulated in the motor cortex of MND patients when compared to normal patients.

Discussion and conclusions: These results indicate that complement activation, leading to increased expression of C5a and its receptors in the hSOD1^{G93A} mice and MND patients has an important role in motor neuron death and may

therefore play a role in the progression of MND. Hence reducing complement-induced inflammation could be an important therapeutic strategy to treat MND.

P112 PRE-SYMPTOMATIC NEUROINFLAMMATION IN ALS: A NOVEL METHOD FOR STUDYING TRANSCRIPTIONAL CHANGES IN MURINE VASCULAR ENDOTHELIUM

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Keywords: endothelium, neuroinflammation, SOD1

Background: Multiple sources of evidence have implicated inflammatory processes in the pathogenesis of ALS. Expression of the mutant protein in neurons or glia alone is insufficient to produce full pathology in the transgenic *SOD1* mouse model of ALS, suggesting that the pathogenesis is non-cell autonomous. Haematogenous leucocytes may gain entry to the CNS and induce microglia to adopt either a pathogenic (M1) or protective (M2) phenotype. The changes that occur at the level of the blood-brain barrier (BBB) and blood-spinal cord barrier (BSCB) that allow leucocytes or inflammatory molecules to enter the CNS, might be a modifiable process in the treatment of human ALS.

Objectives: Through the novel application of an optimized protocol for endothelial cell isolation, we sought to investigate transcriptional changes in an experimental inflammatory model, and in pre-symptomatic transgenic *SOD1* mice.

Methods: A single cell suspension of mouse neural tissue was added to a rabbit CD31 (PECAM) antibody-magnetic bead mixture. Endothelial cells were isolated by exposure to a magnet and a sample of endothelial-depleted and endothelial-enriched brain underwent FACS analysis.

For the inflammatory experiment, mice were injected into the left striatum with either saline, IL-1b (100 ng) or TNF-a (750 ng). RNA was extracted from the endothelial cells removed as described, converted to cDNA, and qPCR performed for the transcripts: IL1b, VCAM-1, ICAM-1, Claudin-5, TLR4 and a_V integrin.

The sensorimotor cortex, brainstem, cervical enlargement and lumbar enlargement from 5 female $SOD1^{G93A}$ and 5 control littermates (80 days $\pm$ 1) were dissected, and endothelial cells extracted. RNA was extracted and converted to cDNA, and PCR performed for the same transcripts.

Results: FACS analysis confirmed isolation of endothelial cells with high purity ($>$97% $CD31^+$). Cell viability was very high, with $<$1% of cells positive for propidium iodide. From the model of experimental inflammation endothelial cells from both injected groups showed higher levels of IL-1b, VCAM-1 and ICAM-1, and lower levels of Claudin-5 compared to saline-injected mice. In the *SOD1* model expression of VCAM-1 and ICAM-1 was increased in the cortex, brainstem and cervical spinal cord, and TLR4 and a_V integrin in the cortex, compared to controls.

Discussion: It was possible to differentiate cytokine-injected from control animals using RT-PCR of the endothelial cell RNA, and to demonstrate inflammation-related transcriptional alterations in the vascular endothelium in pre-symptomatic *SOD1* mice.

Conclusions: Understanding the earliest pathological changes in models of ALS holds promise for developing novel disease biomarkers and drug targets in the human disease.

P113 DYSREGULATED PERIPHERAL IMMUNE RESPONSE EITHER PROMOTES MOTONEURON DEATH OR FAILS TO SUPPORT MOTONEURON SURVIVAL IN AN ALS MOUSE MODEL

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Keywords: SOD1, T cells, axotomy

Background: Amyotrophic lateral sclerosis (ALS) axonal pathology and pre-synaptic deafferentation precede motoneuron (MN) degeneration during disease progression in patients and in an ALS mouse model (mSOD1). Previously in our laboratory, the phenotypic molecular response of wild-type (WT) and pre-symptomatic mSOD1 facial MN (FMN) and surrounding neuropil following facial nerve transection was characterized in a laser microdissection study. The MN gene expression response to axotomy is similar between WT and pre-symptomatic mSOD1 mice geared towards survival and axonal regeneration. However, the non-neuronal cells surrounding mSOD1 MN exhibit a differential expression pattern after axotomy compared to WT, potentially contributing to abnormal neuronal-neuropil cellular interactions to cause the increased MN degeneration. Since we observed a dysregulated microenvironment surrounding MN cell bodies centrally and previously showed that $CD4^+$ T cells are required to maintain WT levels of MN survival and nerve regeneration, we proposed that the microenvironment surrounding the MN axons peripherally may also be dysregulated.

Objective: The present study investigated the neuroprotective functionality of mSOD1 peripheral immune cells in their ability to promote facial nerve regeneration and FMN survival levels.

Methods: First, WT and pre-symptomatic mSOD1 mice received a facial nerve crush axotomy to determine facial nerve regeneration via behavioral functional recovery assessments. All mice were observed daily until complete functional recovery was observed for eye blink reflex, vibrissae orientation, and vibrissae movement. FMN survival levels were assessed at 28 days post-axotomy. Secondly, several immunoreconstitution experiments were conducted using the adoptive transfer of either whole splenocytes or $CD4^+$ T cells isolated from the whole splenocytes into immunodeficient (RAG2 KO) or pre-symptomatic mSOD1 mice. The adoptively transferred cells were injected into the tail vein of the mice 1 week prior to facial nerve transection. The adoptive transfer groups include RAG2 KO mice + mSOD1 splenocytes, mSOD1 mice + WT splenocytes, RAG2 KO mice + WT $CD4^+$ T cells, RAG2 KO mice + mSOD1 $CD4^+$ T cells, and mSOD1 mice + WT $CD4^+$ T cells. FMN survival levels were assessed at 28 days post-axotomy.

Results: Pre-symptomatic mSOD1 mice demonstrated a delayed functional recovery response compared to WT after a facial nerve crush. Unlike WT splenocytes, pre-symptomatic mSOD1 splenocytes are not capable of neuroprotection to rescue the axotomy-induced cell death in immunodeficient mice. However, mSOD1 $CD4^+$ T cells, isolated from the diseased/inflammatory microenvironment and placed in a non-diseased microenvironment, are capable of mediating neuroprotection to a similar level as WT $CD4^+$ T cells.

Conclusions: The peripheral immune response in pre-symptomatic mSOD1 mice resembles that observed in immunodeficient mice following axotomy. Furthermore, the pre-symptomatic mSOD1 peripheral immune microenvironment prevents the activation of neuroprotective $CD4^+$ T cells.

Support: NIH grant NS40433 (KJJ & VMS)

P114 PROGRESSIVE ACCUMULATION OF NERVE/GLIAL ANTIGEN 2 PROTEOGLYCAN IN THE SPINAL CORD WITH MUTANT SOD1-INDUCED NEURODEGENERATION IN RATS

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Keywords: SOD1, CSPG, NG2

Background: Approximately 2% of amyotrophic lateral sclerosis (ALS) cases are linked to mutations in the *Cu/Zn superoxide dismutase* (*SOD1*) gene. Nerve/glial antigen 2 (NG2) is one of the major chondroitin sulfate proteoglycans (CSPG) upregulated after a variety of acute insults in the adult central nervous system (CNS), where they inhibit axonal regeneration and conduction.

Objectives: In order to clarify the role of NG2 proteoglycan under a chronic neurodegeneration such as ALS, we examined a temporal expression of NG2 proteoglycan and the NG2-expressing cell phenotypes in the spinal cord of a transgenic rat model of ALS.

Methods: The expression of NG2 proteoglycan was examined in spinal cord of His46Arg and Gly93Ala mutant *SOD1* transgenic (Tg) rats at pre-symptomatic, early symptomatic, and late symptomatic stages with their age-matched non-transgenic (non-Tg) littermates. Continuous administration of a thymidine analogue bromodeoxyuridine (BrdU) for 7 days labelled newborn cells *in vivo*. After the administration, we performed multiple immunohistochemistry employing anti-NG2 specific antibody and cell-selective markers in the lumbar spinal cord cryosections. The immunofluorescence was digitally captured under confocal laser-scanning microscopy to determine the NG2-expressing cellular phenotype. In addition, we quantified the core protein expression levels by immunoblotting.

Results: In contrast to non-Tg rats, the 2 lines of Tg rats showed a significant and progressive accumulation of NG2 proteoglycan at the site of neurodegeneration in the ventral spinal cord. The multiple immunohistochemistry with cell-selective markers revealed that activated microglial cells, microvascular endothelial cells, and oligodendrocyte progenitor cells were suggested to constitute the NG2-expressing cells. In the gray matter, especially in the ventral horn, microglial cells were the major components of NG2-espressing cells and they often aggregated with the phagocytic features.

Discussion and conclusions: In the present study, we revealed a progressive accumulation of NG2 proteoglycan at the site of motor neuron degeneration in the rat ALS model. Recent reports have shown that the NG2-expressing microglial cells are involved in promoting neuroinflammation, and both the NG2 itself and inflammation can counteract the regenerative process in the damaged CNS. Therefore, NG2-expressing cells such as activated microglial cells may be considered as a potential therapeutic target in ALS.

P115 PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE DEFICIENCY DELAYS TONGUE MOTOR FUNCTION DEFICIT AND PROLONGS SURVIVAL IN THE SOD1-G93A MOUSE MODEL OF ALS

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Keywords: PACAP, neuroprotection, microglia

Background: Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide with pleitropic functions, and expressed by many neurons throughout the central nervous system, including motoneurons. It has been reported that PACAP has neuroprotective properties, e.g. in preventing ischemic delayed neuronal cell death, and that it is a general anti-inflammatory factor, both in innate and adaptive immunity. Recently, it was shown that PACAP is able to protect rat motoneurons against glutamate-induced excitotoxicity *in vitro* (1), a pathomechanism discussed for ALS. Moreover, PAC1, the specific PACAP receptor, is expressed by astrocytes throughout the central nervous system, suggesting that astrocytes could also be a target of PACAP action in the process of non cell-autonomous motoneuron degeneration.

Objectives: We asked if a genetically induced PACAP deficiency (PACAP knockout) affects disease onset, progression, and motor functions in SOD1-G93A mice, the most frequently used mouse model of ALS.

Methods: We crossbred a PACAP-deficient mouse strain into the SOD1-G93A mouse model. Starting at postnatal day (P) 49, all mice (SOD1:PACAP-/-; SOD1/PACAP+/+; wt:PACAP-/-; and wt:PACAP+/+, 15-20 animals per group) were clinically monitored on a weekly basis, including measurement of body weight, paw grip endurance (PaGE) and licking motor tests, and finally survival.

Results: SOD1:PACAP-/- mice showed prolonged survival (142 +/- 12 days) compared to SOD1:PACAP+/+ (131 +/- 7 days, $p = 0{,}002$). While both groups showed no differences in body weight loss and PaGE performance, tongue motor deficits, determined by licking frequency, were delayed in SOD1:PACAP-/- mice (mean onset at P112) compared to SOD1:PACAP+/+ (mean onset at P105; $p = 0.022$). In addition, licking performance of SOD1:PACAP-/- also stayed significantly better than in SOD1:PACAP+/+ until end-stage.

Discussion and conclusions: Since PACAP deficiency prolonged survival of SOD1-G93A mice that was accompanied by a partially better motor performance compared to PACAP competent littermates, we conclude that PACAP may not generally be neuroprotective *in vivo*, at least in this ALS

model. Differences to *in vitro* and *in vivo* observations made by others may be explained with additional deleterious effects of PACAP on innate and/or adaptive immunity, both contributing to disease. Since these effects could exceed neuroprotective autocrine pathways and be more important for ALS pathogenesis, they are currently under investigation.

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P116 SITE-SPECIFIC EXCITOTOXIN EXPOSURE *IN VIVO* LEADS TO NEURONAL EXCITOTOXICITY AND AXONAL DYSFUNCTION

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Keywords: excitotoxicity, axonal degeneration, axonal dysfunction

Background: Despite recent advances, there still remain significant gaps in our current understanding of the mechanisms underlying axon degeneration in amyotrophic lateral sclerosis (ALS). ALS is likely to be a multifactorial disease of neuronal dysfunction and loss, however, recent investigations indicate that axonal dysfunction, prior to cell loss, may be the causative factor of the initial symptoms of ALS. Furthermore, it has been demonstrated in mouse models of familial ALS, other motor neuron diseases and, more recently, in studies of early human ALS, that distal axonal degeneration may occur before the onset of disease symptoms.

Objectives: Our investigations are focused on determining the degenerative changes underlying ALS-like axonopathy by using site-specific excitotoxic insults *in vivo.* We have developed a site-specific mouse model of excitotoxicity utilising osmotic minipumps (Alzet, model 1004), which will enable us to investigate the primary site of excitotoxic damage related to axonal pathology and ALS-like functional decline *in vivo*.

Methods: A constant and chronic infusion of Kanic acid (1-5mM, in cortex buffer) was delivered to the subarachnoid space of the lumbar region (L4-5) of C57/Bl6 mice and transgenic mice which express yellow fluorescent protein in a subset of motor neurons on a C57/BL6 background. Time and age matched controls were administered a constant infusion of cortex buffer. Fluro Ruby (2μM) was infused to determine the distribution of the Kanic acid. Animals were kept to a maximum of four weeks and all mice were terminally anaesthetised, transcardially perfused with 4% paraformaldehyde and then processes for immunohistochemistry to determine pathological changes occurring at the NMJ (distal axon), sciatic nerve (axon) and spinal cord (cell body and proximal axon).

Results: Fluoro Ruby labelling was present throughout cells within the subarachnoid space in L4-5 and a small number of neurons within the ventral horn, indicating a targeted delivery can be achieved with the osmotic pumps.

Discussion and conclusions: The data obtained by these experiments will enable us to investigate the primary site of excitotoxic damage related to axonal pathology and ALS-like functional decline *in vivo*. Identifying the site of the initial effects of excitotoxicity will identify mechanisms of distal axon degeneration that may provide novel therapeutic targets directed at axon protection.

P117 PULSATILE GROWTH HORMONE SECRETION IN THE SOD1G93A MOUSE MODEL OF ALS RESEMBLES GROWTH HORMONE DEFICIENCY IN ALS PATIENTS

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Keywords: growth hormone, endocrinology, SOD1G93A mouse

Background: The endocrine control of energy homeostasis is disrupted early in Amyotrophic Lateral Sclerosis (ALS), suggesting that this might contribute to ALS pathogenesis. ALS patients have increased basal energy expenditure, lean body mass index and muscle mitochondria abnormalities (1-5). These factors are thought to contribute to muscle wasting in ALS. Growth hormone (GH) is an anabolic hormone that aids in the development and maintenance of healthy muscle mass. Therefore, GH deficiency in ALS may also promote muscle wasting and be a consequence of the persistent hypermetabolic state in ALS. The mechanistic contribution to and effects of the disrupted GH axis in the loss of muscle mass in ALS however remains unknown. To further investigate these mechanisms we must first confirm that disruptions in the GH axis observed in ALS patients (6) also occurs in animal models that are used to study ALS.

Objective: To characterize the pattern of GH secretion in a transgenic mouse carrying the human SOD1 mutation.

Methods: Male wild-type and SOD1G93A transgenic mice were studied at the end-stage of disease (150-180 days). To determine one-off measures of plasma GH, blood was collected by cardiac puncture between 1530hrs and 1700hrs. To assess pulsatile GH secretion in mice, tail-tip whole blood samples (4μl) were collected consecutively over a 6hr period at 10min intervals starting at 0630hrs. An in-house GH ELISA was used to determine GH concentration. Data were analyzed by deconvolution analysis for properties associated with the pulsatile pattern of GH secretion.

Results: Analysis of single blood samples show that SOD1G93A end-stage mice and wild-type age-matched controls expressed similar plasma GH ($n \geq 12$, $p = 0.9449$, t-test). By contrast, analysis of pulsatile GH secretion in samples collected over a 6hr period confirms a significant reduction in the total amount of GH secreted over the sampling period ($n = 8$ wild-type, $n = 7$ SOD1G93A). Furthermore, we observed a disruption in the pulsatile pattern of GH secretion. This was characterized by an overall decrease in the peak amplitude, and a loss of regularity of GH pulses. These observations suggest a disruption in the regulation of GH secretion in SOD1G93A mice at the end-stage of disease.

Discussion and conclusions: We report the first definitive account of disrupted GH secretion in a transgenic mouse model of ALS. Disrupted GH secretion in the SOD1G93A mouse closely resembles that seen in ALS patients. Defining the time course, causes and consequences of changes in GH secretion may lead to a greater understanding of what drives the onset and rate of progression of ALS symptoms, and can potentially provide new biomarkers and therapeutic strategies for ALS.

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P118 SYNERGY OF ERYTHROPOIETIN AND INSULIN-LIKE GROWTH FACTOR-1 CAN DETERIORATE CU,ZN-SUPEROXIDE DISMUTASE MUTANT AMYTORPHIC LATERAL SCLEROSIS VIA ACTIVATED MAMMALIAN TARGET OF RAPAMYCIN-INDUCED AUTOPHAGY INHIBITION

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Keywords: erythropoietin, insulin-like growth factor-1, autophagy inhibition

Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease presenting progressive motor weakness and respiratory failure within a few years from symptom onset. Many theories about pathogenesis are proposed including misfolded protein aggregation, mitochondrial dysfunction and oxidative cytotoxicity but exact pathogenic mechanisms are still not identified. Erythropoietin (EPO) and insulin-like growth factor I (IGF-1), show neuroprotective effects individually and have demonstrated to have synergistic neuroprotective properties in Alzheimer's dementia and Parkinson's disease when delivered simultaneously by activating phosphoinositide 3-kinase (PI3K) and Akt (protein kinase B) pathways. The possible limitation of their high-dose therapy causing systemic side-effect can be averted by detouring blood brain barrier (BBB) via delivering them through olfactory epithelium deficient of BBB. Autophagy is linked to neurodegenerative diseases as one mechanism for removing misfolded proteins. Target of rapamycin (mTOR) pathway, critical negative regulator of autophagy is located at downstream of PI3K/Akt pathway, of which activation could affect autophagy. However, their relationship to autophagy has not yet been elucidated. In this study, we evaluated the synergistic effect of EPO and IGF-1 simultaneously delivered in ALS using G93A Cu, Zn-superoxide dismutase (SOD1) mutant transgenic mice.

Method: We administered EPO and IGF-1 simultaneously to G93A SOD1 transgenic mice by transnasal delivery. Rotarod performance and survival analysis were assessed. Western blot and real-time PCR were done in order to investigate the drug mechanism. We then evaluated the effect of IGF-1 and EPO on the light chain 3 (LC3) dots representing autophagosomes by adopting GFP-LC3 transfected stable cell-line. We established NSC-34 cell line stably expressing human G93A SOD1 mutant, and assayed cytotoxicity.

Results: We found that EPO and IGF-treated G93A transgenic mice showed significantly poor performance on the rotarod task and earlier death compared with the mice treated with placebo. Western blot using antibodies against LC3, phosphorylated mTOR, and substrates of mTOR showed reduction of ratio of LC3-II /LC-I, and the activation of mTOR pathway. In-vivo imaging of GFP-LC3 expressing cells, rapamycin-induced LC3 dots were blocked by simultaneous treatment of IGF-1 and EPO. At the cell death assay, simultaneous treatment of IGF-1 and EPO induces more cell death in thapsigargin-induced cell death.

Conclusion: These findings showed that EPO and IGF-1 accelerate disease progression in G93A SOD1 transgenic mice compared with the placebo-treated group. The simultaneous treatment of EPO and IGF-1 activates mTOR pathway resulted in the decrease of autophagy. It could be a possible explanation of the deterioration caused by EPO and IGF-1, since autophagy is thought to remove mutant SOD1 aggregation. This EPO and IGF-1 mediated autophagy inhibition means that mTOR can be activated by the synergistic activation of PI3K/Akt pathway. It implicates that the inter-relationship between PI3K/Akt and mTOR pathways should be considered thoughtfully in order to develop new drug to target these pathways.

P119 DYSREGULATION OF INTRACELLULAR COPPER HOMEOSTASIS IS COMMON FOR DISMUTASE ACTIVE AND INACTIVE FORM OF SOD1 MUTANTS

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Keywords: intracellular copper homeostasis, TTM, dismutase active and inactive SOD1 mutants

Introduction: Mutations in the SOD1 gene cause familial ALS through a gain of toxic property. We have recently shown that dysregulation of intracellular copper homeostasis contributed to disease progression in a mouse model of ALS, which overexpresses the dismutase active G93A mutant SOD1 (1). However, it is unknown whether dysregulation of intracellular copper homeostasis is common for both dismutase active and inactive SOD1 mutants.

Objectives: The aim of the present study was to elucidate whether dysregulation of intracellular copper homeostasis could be a common feature in mutant SOD1 toxicity.

Methods: We used four different transgenic mouse strains overexpressing human SOD1 with the ALS-causing mutations: $SOD1^{G93A}$, $SOD1^{D90A}$, $SOD1^{G85R}$ and $SOD1^{G127insTGGG}$ ($SOD1^{G127X}$). For each mutant SOD1 mouse model, spinal cords and brain were harvested from three end-stage mice of each genotype. C57BL/6J mice were used as controls.

The copper levels in the spinal cords were measured using inductively coupled plasma mass spectrometry. Because intracellular copper homeostasis is tightly regulated by intracellular copper trafficking-related proteins, we analyzed the expression level of the proteins using Western blot.

For treatment with an intracellular copper chelator, ammonium tetrathiomolybdate (TTM), $SOD1^{G93A}$ mice were randomly assigned to receive a daily intraperitoneal administration with TTM (5 mg/kg) or phosphate buffered saline. Treatment was started at 13 weeks of age, after the $SOD1^{G93A}$ mice began to exhibit ALS-like symptoms.

Results: Incorporation of copper ion into the copper-binding site is essential for SOD1 dismutase activity. Among mutant SOD1 mice that we used here, the $SOD1^{G93A}$ and $SOD1^{D90A}$ retain dismutase activity, whereas the $SOD1^{G85R}$ and $SOD1^{G127X}$ lack the activity due to insufficient copper ligation and are also present at very low levels in the CNS (2). We found that spinal copper levels were significantly increased in dismutase active form of mutant SOD1s mice. Interestingly, elevated copper levels were also observed in the inactive forms even though copper ions are not bound to $SOD1^{G85R}$ and $SOD1^{G127X}$.

Western blot analysis showed that all mutant SOD1s shifted intracellular copper homeostasis toward copper accumulation: Ctr1, a copper uptake transporter, was significantly increased, whereas Atp7a, a copper efflux pump, was decreased. No changes were found in the brains.

Treatment with TTM, starting at a symptomatic stage, significantly prolonged the mean survival of $SOD1^{G93A}$ mice by 11%. Similarly, treatment with TTM dramatically slowed disease progression by 40%. TTM restored the elevated spinal copper ions to a normal level in $SOD1^{G93A}$ mice.

Conclusions: Enzymatically active and inactive SOD1 mutants share dysregulation of intracellular copper homeostasis. TTM might be an attractive candidate drug for therapy to ALS patients with SOD1 mutations.

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P120 TDP-43 PATHOLOGY IN MICE EXPRESSING A VERY LOW COPY NUMBER OF THE MUTANT HUMAN G93A SOD1 GENE WITH A VERY SLOW DISEASE PROGRESSION

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Keywords: TDP43, SOD1, copy number

Background: Transgenic (Tg) mice expressing multiple copies of the human mutant SOD1 gene develop motor neuron (MN) pathology and clinical symptoms that are similar to patients with ALS/MND. In mice with 24 copies of the transgene, MN pathology is evident as early as 30d old and degeneration proceeds rapidly. This time course makes it difficult to discern disease onset, and may not reflect events in human MN degeneration. We have developed Tg mice (VLE mice) expressing a very low number (4-5) of copies of the G93ASOD1 transgene. These mice do not show clinical signs of MN disease until 650d old, if at all, providing the opportunity to more clearly discern both the sequence and nature of the pathologic changes in the mouse model and compare it to human ALS.

Objective: To determine whether pathological changes in TDP-43 and related molecules characteristic of human ALS, which have not been detectible in the rapidly progressing mouse model, are present in very slowly progressing mice with the SOD1 mutation.

Methods: VLE mice were compared to typical G93ASOD1 Tg mice (HE mice) expressing a high number of copies (24) of the transgene. All are on the B6SJL/F1 background. The number of gene copies was confirmed by qPCR. Male and female VLE mice at approximately 250, 500 and 750d old and HE mice at 90 and 120 days old were examined along with non-transgenic littermates. Paraformaldehyde-perfused spinal cords were embedded in paraffin, sectioned, and immunostained with an antibody to TDP-43 (Proteintech, 1:500). Staining patterns in the MNs in the ventral horns of lumbosacral segments were determined. MNs were easily identifiable by their position and large (>30 μm) cell bodies.

Results: TDP-43 labeling in young (90 and 120 day old) HE mice was largely confined to the nucleus, as previously observed. Labeling was similar in 250 day old VLE mice, an age at which motor neuron loss is not yet significant, although some cytoplasmic labeling was beginning to become evident. In older VLE mice, TDP-43 is distinctly distributed in cytoplasmic accumulations.

Discussion and conclusions: Redistribution of TDP-43 from the nucleus into cytoplasmic accumulations is observed in human ALS spinal cord MNs, in both FALS and SALS cases, but has not been established in the SOD1 mouse model. We hypothesized that the rapid degeneration of MNs in these mice may obscure these cellular changes characteristic of human ALS. Examination of TDP-43 distribution in MNs of VLE mice, in which degeneration proceeds at a very slow rate, revealed cellular changes more in line with those observed in human ALS. This slowly-progressing model of MN degeneration may be more relevant to cellular pathological changes in human ALS.

P121 DEVELOPMENTAL ROLE OF RNA PROCESSING PROTEINS IN NEURODEGENERATION

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Keywords: zebrafish, RNA processing proteins, in vivo imaging

Background: The most recent advances in understanding ALS pathology come from the study of the RNA processing proteins TDP-43 and FUS. These genes were found to be mutated in both familiar and sporadic ALS and the proteins found in aggregates associated with the disease, but so far their mode of action remains unknown.

Objectives: Our work intends to unravel the function of TDP-43 and FUS by establishing the zebrafish as a model for neurodegeneration mediated by these RNA processing proteins.

Methods: We are performing gain and loss of function studies of zebrafish homologues of TDP-43 (tardbp and tardbpl) and FUS by micro-injecting either RNA or DNA (gain), or antisense morpholinos (MO, loss). These manipulations allow us to address the developmental role of these proteins. We are also overexpressing the human forms of the wildtype (wt) genes and several different mutations found in ALS patients. Loss-of-function (LoF) of the zebrafish proteins combined with the gain-of-function (GoF) of nuclear and cytoplasmic human forms reveals their relative contribution to the toxicity of these proteins.

Using fluorescent tag proteins we are also performing live imaging to analyze the intracellular protein dynamics in real time.

Results: Embryos lacking tardbp and tardbpl function consistently showed pericardial oedema, poor circulation, slow mobility, and reaction to touch from 3 days post fertilization (dpf) onwards. Motor neuron axons are shorter, with abnormal branching.

In the fus LoF, the embryos also have pericardial oedema, hydrocephaly and bent tail. Embryos at 3dpf still react to touch and are able to swim, having mild locomotion problems compared to wt. The motor neuron axons are unaffected.

Triple knockdown (tardbp + tardbpl + fus) show a more severe phenotype than the single or double combinations. The embryos are immobile and do not react to touch. The motor neuron axons show abnormal branching and migratory problems; ectopic cell bodies are visible adjacent to the spinal cord.

The GoF of tardbp and fus both have similar phenotypes; shorter and bent trunk, small eyes and head, cell death. The locomotion and touch response are not affected.

Rescue of LoF phenotypes by overexpressing different human constructs for TDP-43 and FUS are underway.

Discussion and conclusion: The zebrafish is becoming a powerful model for studying the function of RNA processing proteins in neurodegeneration. The LoF studies show a phenotype in the locomotion in response to touch. The triple knockdown suggests a functional interaction between tardbp/tardbpl and fus. We are now currently analyzing the factors behind this phenotype, looking at the activity, stability and connectivity of the motor neurons and performing RNA profiling studies.

The GoF studies are encouraging and will help elucidate the function of these proteins during early development. The information gained in these studies will contribute to the understanding of ALS pathology and ultimately deliver potential therapeutic targets.

P122 LIVE IMAGING OF MOTOR AXONS IN ZEBRAFISH EMBRYOS EXPRESSING AN ALS-RELATED MUTATION OF HUMAN TARDBP

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Keywords: in-vivo imaging, motor axon, transgenic zebrafish

Background: Amyotrophic lateral sclerosis (ALS) is associated with loss of motor neurons, however the origin of the neural dysfunction remains unknown. Previous studies suggest that deficits in axon function precede motor neuron death.

Objectives: To study axonal morphology in the context of ALS we followed the motor axon arbor organization and growth, *in-vivo,* in zebrafish embryos expressing GFP under the motor neuron promoter HB9 and expressing the sporadic and familial ALS-related mutation G348C of human *TARDBP* under the inducible heat-shock *Hsp70* promoter.

Methods: Trangenic zebrafish embryos were subjected to heat-shock at 24h post fertilization at 38.5°C for 30 minutes (sufficient to induce ubiquitous expression) and imaged 2-4 hours later, every 2 minutes for a total of 30 minutes using a spinning disk confocal microscope. The stacked images of the motor axons were then 3D reconstructed for each time point using Imaris software (Bitplane, USA). The 3D reconstruction provided information about main axon and secondary neurite length, density and motility. The results from transgenic embryos were compared to data obtained from control embryos including non-transgenic siblings that were heat-shocked and transgenic and non transgenic siblings that were not heat-shocked.

Results: Transgenic embryos imaged as soon as 2h hours after the heat-shock had hyper branched primary axons compared to control embryos which had one main axon. These results confirm data obtained previously (1) from embryos injected with the human mRNA carrying G348C mutation and fixed 48h post fertilization. In addition, the number of secondary neurites was reduced in the mutant, but their length was similar to that of the controls. The terminal endings of the main branches in control embryos were quite stable but were more motile in mutants, suggesting unstable innervation by supernumerary mutant axon branches.

Discussion and conclusions: Our study is first to follow axon structure and dynamics *in-vivo* in the context of ALS and our preliminary results suggest that the motor neuron axon deficits occurs soon after the expression of the mutated TARDBP protein, before motor neuron death.

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P123 DEFECTIVE NEUROMUSCULAR TRANSMISSION IN ZEBRAFISH EXPRESSING HUMAN TARDBP (TDP-43) WITH A MUTATION RELATED TO ALS

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Keywords: zebrafish, TDP-43, electrophysiology

Background: Mutations in the *TARDBP* gene encoding TDP-43 have been found in ALS and FTLD patients. Although several animal models for these TDP-43 mutations have been described, none have characterized the pathophysiological deficits that underlie the resulting phenotype.

Objectives: To advance our understanding of the neurophysiological deficits resulting from mutations in the TARDBP gene encoding TDP-43.

Methods: We used a zebrafish model previously described by our lab (1) in which we transiently expressed mutant human $TARDBP^{G348C}$ as well as wildtype $TARDBP^{WT}$ mRNA in zebrafish larvae. Standard Immunohistochemistry and patch-clamp recordings were performed on muscle and motor neurons in larvae aged 48 hrs.

Results: Following over expression of mutant but not WT TDP-43 we observed specific deficits at the neuromuscular junction (NMJ). Immunohistochemistry revealed hyper-branched motor neuron endings with AChR clusters at NMJs in fish expressing $TARDBP^{G348C}$ but not $TARDBP^{WT}$ or WT fish. We next examined spontaneous miniature endplate currents (mEPCs) related to the release of single transmitter quanta at the NMJ. Two striking features were observed: the amplitude of mEPCs was significantly larger and occurred at a lower frequency in fish expressing mutant $TARDBP^{G348C}$ but not WT *TARDBP*. We next evoked swimming-related activity and observed normal rhythmic EPCs at NMJs from

fish expressing *TARDBP*^{WT}. In contrast, fish expressing *TARDBP*^{G348C} displayed a slower frequency of rhythmic EPCs with shorter bouts of swimming-related activity. In paired recordings action potentials were elicited in motor neurons while recording EPCs in the muscle. We observed an impairment in the fidelity of sustained synaptic transmission.

Discussion and conclusions: These data represent the first electrophysiological description of *TARBP*-related mutations and indicate that over branched motor neurons form NMJs each with exaggerated spontaneous release and yet overall weaker sustained release at the NMJ.

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P124 TDP-43 TRANSGENIC *C.ELEGANS* DEVELOPED MOTOR DYSFUNCTION CHARACTERISTIC OF ALS

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Keywords: TDP-43, C.elegans

Objective: To deliver the expression plasmid:unc-51/hTDP-43 by microinjection to the gonad of *C.elegans* to establish a TDP-43 ALS model and to investigate the mechanism of TDP-43 motor neurotoxicity, including dissection of which molecular features of TDP-43 pathology cause motor neuron degeneration.

Methods: To generate transgenes expressing human TDP-43 driven by a pan-neuronal unc-51 promoter, which were Punc-51::TDP-43(WT) Punc-51::TDP-43(G348C), Punc-51::TDP-43(S292A), and Punc-51::TDP-43 (CTFs) respectively. The transgenes described above were microinjected into hermaphrodite gonads of N2 worm at a concentration of 50-100μg/ul to generate multiple extrachomosomal lines based on the green fluorescent marker. The locomotion of control and transgenic worms was assayed by picking 10-20 L4 or adult worms onto 100 mm nematode growth medium (NGM) agar plates spread with a uniform bacterial lawn. Worm locations were then recorded at the indicated time points, and linear distances from the starting position were measured. And the neurodegeneration assays were performed that timed egg lays were arranged to produce synchronized populations 8, 24, and 48 h of age. Live worms were placed on a 3% agarose pad containing 0.01% sodium azide to immobilize the worms. Worms were imaged under fluorescence microscopy and scored for number of dorsal motor neurons, and gaps in the dorsal nerve cord. Finally, this ALS model was examined by Western blot. In order to analyze the cellular localization of TDP-43, immunocytochemistry was performed.

Results: By gene clone, we successfully constructed hTDP-43 expressive vectors, they are pU51P::hTDP-43(WT), pU51P::hTDP-43(G348C),pU51P::hTDP-43(S292A), pU51P::hTDP-43(CTFs) respectively. By microinjection and offspring screening, we obtained the stable inherited ALS model of TDP-43 proteinopathy. The biological characteristics of this ALS model: typical motor dysfunction; the degeneration and loss of motorneuron synapses; and TDP-43 proteins aggregated in the nucleus of neurons.

Conclusions: Use of *C.elegans* as a model system permits unbiased approaches to genetically manipulate a model of human neurodegenerative diseases. This TDP-43 ALS model is likewise tractable for genome-wide forward and reversed genetic screens, which will allow identification of novel modifiers of TDP-43.

P125 SPATIAL CHARACTERIZATION OF THE MOTOR NEURON COLUMNS SUPPLYING THE RAT FORELIMB

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Keywords: rat models, motor neurons, cervical spinal cord

Background: Rats can generate a rich array of forepaw and forelimb movements that are similar to those produced by humans, therefore making them attractive models to validate therapeutic intervention for the recovery of motor control. We are currently exploring strategies using virus to deliver therapeutic genes to specific populations of motor neurons in the rat. This can be achieved via intramuscular injections of viral vectors and subsequent retrograde transport and expression of the therapeutic gene in targeted motor neuron populations. In order to achieve this, knowledge regarding the precise relationship between different muscles of the forelimb and the location of motor neurons that innervate them must be first established.

Objectives: This study aims to examine the details of the arrangement of motor neurons that supply the rat forelimb.

Method: Eleven upper limb muscles from the rat were selected. The muscle motor end plates were visualized by means of acetylcholinesterase histochemistry and this information was then used to create a motor end plate map of the forelimb. This map was used as a guide to perform multiple injections of fluorescent retrograde tracers (Fluoro-Gold and Fluoro-emerald) along the motor end plate region of the selected forelimb muscles. 12-14 days later, the rats were perfused intra-cardially and the spinal cord segments of interest were dissected out, sectioned and analysed under epifluorescence. For each muscle, the positively labelled motor neurons were plotted on a schematic reconstruction of the spinal cord. The individual plots were then stacked in order to create motor neuron maps in all axes.

Results: This tract-tracing analysis confirmed that motor neurons innervating the rat forelimb are arranged in columns that span across multiple spinal cord segments. Individual motor columns exhibit a substantial degree of overlap with other motor columns in all planes (i.e. rostro-caudal, dorso-ventral and medio-lateral axes).

Discussion and conclusions: This anatomical investigation supports previous observations that, although discrete, some of the motor neuron columns lying in the cervical aspect of the rat spinal cord are inter-digitized. The length of these columns, and hence the overlap between them, appears to be greater than previously reported, particularly within the uppermost segments of the brachial plexus. This map constitutes a valuable guide for the selection of appropriate muscle(s) for the delivery of therapeutic genes into specific segments of the cervical spinal cord. The organization of the motor neurons supplying the rat forelimb may have significant applications for the development of therapies in rat models of forelimb dysfunctions including models of motor neuron diseases.

P126 A REDUCED REQUIREMENT FOR SMN, THE SPINAL MUSCULAR ATROPHY PROTEIN, DURING ADULT LIFE

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Keywords: spinal muscular atrophy, SMN protein, therapeutic window

Prior observations of the effects of reduced Survival Motor Neuron (SMN), the underlying cause of the childhood disorder, Spinal Muscular Atrophy (SMA), have been made largely in embryos and neonates. The requirement for the protein during adult life has not been investigated. We and others have shown that reduced protein arrests the development of one component of the neuromuscular system - the neuromuscular synapse, raising the possibility that once the synapse is mature, depleting protein, e.g., in adults may not adversely affect the organism. To test this possibility, we have generated model mice harboring a tamoxifen-responsive *Smn* allele (SMN^{F7}) and human *SMN2* transgene and ubiquitously reduced the SMN protein at different time points during postnatal life.

Our results demonstrate, for the first time, that the requirement for SMN is higher in young mice than in adults. Surprisingly, depleting protein in adults carrying 2 copies of the human *SMN2* (*Cre-ER*$^{+/-}$;$SMN^{F7/-}$;$SMN2^{+/+}$) to severe SMA levels appeared not to result in an overt phenotype whereas a similar reduction in young animals caused progressive muscle weakness.

These data suggest that there is a window of time in postnatal life during which wild-type levels of the SMN protein are required for the normal development of the neuromuscular and/or other systems, following which cellular demand for the protein drops. An important implication is that infants diagnosed with SMA in a timely manner may not require chronic treatment with SMN restoring agents and thus the prospect of possible adverse, drug-related side-effects. Rather, a limited treatment regimen during a critical window of time could be sufficient to allow the proper development of the neuromuscular system and prevent an SMA phenotype from eventually developing. Further defining the window of time for SMN requirement and correlating it with the development of various organ systems will inform our understanding of the precise cellular site(s) of action of the protein in health and disease and thus contribute to effective future treatments for SMA patients.

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THEME 8 *IN VITRO* EXPERIMENTAL MODELS

P127 DEVELOPMENT OF AN *IN VITRO* MODEL OF HUMAN MOTOR NEURONS

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Keywords: primary cultures, human motor neurons

Background: Primary cultures of human primary motor neuron (MN) represent a unique tool to study cell characteristics and properties, neurotoxicity, and neuroprotection. This model can also be used to assess molecules of clinical relevance on MN. We have developed a new technique using a combination of centrifugation and gradient density separation process from foetal spinal cord to obtain primary cultures of highly purified human foetal MN.

Methods: We have isolated motor neurons from the human foetal spinal cord (16-20 weeks of gestation). At day 3 *in vitro* (DIV), 1-β-D arabino furanosylcytosine (Ara-C) was used to reduce non-neuronal cell growth. The purity of MN cultures was then assessed by immunocytochemistry for $p75^{NTR}$, SMI-32 markers and cell counting. Following dissociation of spinal cords and separation by gradient centrifugation, cells were analyzed 7 days after plating by immunocytochemistry using a labeling with SMI-32 antibody and DAPI staining for the nuclei. This high purity was confirmed by flow cytometry quantification of MN purity following labeling with anti-$p75^{NTR}$.

Results: We have obtained highly purified ($>85\%$) primary culture of human MN. The percentage of SMI-32 + and $p75^{NTR}$ + MN relative to the number of cell nuclei was 86.17 ± 2.78 and 84.74 ± 3.26 when treated with Ara-C.

Conclusions: We have successfully optimized a new method to obtain highly purified primary cultures of human fetal MN. This tool will be very important to study neurotoxicity and neuroprotection and to assess therapeutic strategies for MND.

P128 ESTABLISHMENT OF HUMAN IMMORTAL MESENCHYMAL STEM CELLS SECRETING MULTIPLE TROPHIC FACTORS USING A HUMAN ARTIFICIAL CHROMOSOME

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Keywords: mesenchymal stem cell, human artificial chromosome, gene therapy

Purpose: The administration of neurotrophic factors or growth factors is a hopeful strategy for the treatment of amyotrophic lateral sclerosis (ALS). Glial cell line-derived neurotrophic factor (GDNF), insulin-like growth factor (IGF-1), and hepatocyte growth factor (HGF) show encouraging outcomes in animal experiments. None, however, has been efficacious in human clinical trials. It is possible that a cocktail of several trophic factors could exert a mutually potentiating effect to alleviate the progression of ALS. Meanwhile cell transplantation, e.g., mesenchymal stem cell (MSC) transfer, is also promising, but it appears not to be potent enough for clinical application. It would therefore be of great interest, if technically possible, to obtain stem cells which expressed multiple trophic factors known to be beneficial for ALS. Here, we pursued that concept using a human artificial chromosome (HAC) system.

Method: A P1-derived artificial chromosome (PAC) vector was constructed with GDNF, IGF-1, and HGF genes, as well as green fluorescent protein (GFP) and luciferase genes (a total of 41,247 base pairs). It was transferred by microcell-mediated chromosome transfer (MMCT) into Chinese Hamster Ovary (CHO) hybrid cells containing the HAC with a loxP system. Trophic factors and markers genes were transferred into the HAC vector in CHO cells using Cre-loxP mediated chromosome translocation. From the CHO hybrids, the HAC vector was further transferred by MMCT to human immortalized MSCs (hiMSCs). The resultant clones were analyzed for the expression of trophic factors by ELISA studies of cultured media, luciferase assays of the lysates of hiMSC clones, and the signal intensities of GFP by fluorescence microscopy.

Results: Of 41 chromosomes transferred and CHO clones analyzed, 13 clones retained the entire DNA sequence of PAC origin (31.7%). Among them, two clones retained the genes of interest on the HAC, not on CHO chromosomes. During MMCT from the CHO hybrids to hiMSCs, 30 clones out of 46 retained the entire PAC-origin DNA sequence (65.2%). RT-PCR revealed two clones that highly expressed mRNAs of the introduced genes. ELISAs, luciferase assays, and microscopic observations for GFP revealed a single clone named

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hiMSC 4pacG3-31, which was suitable for transplantation in the ALS mouse model.

Conclusions: We obtained a hiMSC clone that stably expressed high levels of GDNF, IGF-1 and HGF. This clone also enables us to histologically track cell dynamics after transplantation by GFP and *in vivo* by luciferase. Using this clone, we are now undertaking experimental treatment of $SOD1^{G93A}$ transgenic ALS model mice.

P129 INDUCE DIFFERENTIATION OF MOTOR NEURON-LIKE CELLS FROM HUMAN ADIPOSE-DERIVED STEM CELLS WITH RETINOIC ACID AND SONIC HEDGEHOG *IN VITRO*

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Keywords: motor neuron cell-like cells, human adipose-derived stem cells, retinoic acid, sonic hedgehog

Background: The adipose-derived stem cell (ADSC) might possess the ability to differentiate into neuronal-like cells, however, there was no way to differentiate ADSCs into more specialized subtypes of neurons *in vitro* and *in vivo*. The non-specific cells are often the source of aberrant tissue formation in transplant therapy.

Objective: To test whether retinoic acid (RA) and sonic hedgehog (Shh) have the ability to induce human adipose-derived stem cell (hADSCs) differentiation into the characteristics of motor neurons in the central nervous system.

Methods: The hADSCs were plated on plastic culture dishes at 1-104cells/cm2 and incubated for 2 days. At preconfluence, culture medium was replaced with preinduction medium composed of DF12, 20% of fetal bovine serum, 10 ng/ml of fibroblast growth factor 2 , 2% B27, 250 mM of isobutylmethylxanthine, and 100 mM of 2-mercaptoethanol. Then, the dishes were incubated for 6 h. After that, induction medium composed of DF12, 0.2% B27, 0.01 mM of all-trans RA, and 100 ng/ml of Shh was used. After 1 week, the induced medium was replaced with another one, which contained DF12, 0.2% B27, 200 ng/ml of vitamin C, 100 ng/ml of brain-derived neurotrophic factor, and 100 ng/ml of glial cell-derived neurotrophic factor. For immunocytochemistry test, the cell nuclei were labeled with Hochest. Anti-glial fibrillary acidic protein (GFAP), anti-nestin, anti-myosin heavy chain, anti-b-III-tubulin and anti-choline acetyltransferase were added. Cells were examined with a fluorescence microscope. RT-PCR was performed to compare the expression levels of Olig2, Nkx2.2, Pax6, Hb9, HoxC8, and Otx2.

Results: As early as 6 h after induction, hADSCs were changed toward neuronal morphology. After induction, hADSCs showed positive immunocytochemical staining for b-III-tubulin, choline acetyltransferase, and neuron-specific enolase. Reverse-transcriptase polymerase chain reaction characterization indicated that cells differentiated from hADSCs were restricted to the ventral spinal fate (Nkx2.2, Pax6, Hb9, and Olig2).

Conclusion: When induced by RA and Shh, hADSCs could be differentiated into motor neuron-like cells.

P130 MURINE EOC13 MICROGLIA EXPRESS FUNCTIONAL P2X7 RECEPTORS: POTENTIAL MODEL FOR AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: P2X7, microglia, inflammation

Background: The P2X7 purinergic receptor is a trimeric ATP-gated cation channel expressed on leukocytes including microglia. Stimulation of this receptor results in the uptake of organic ions including ethidium and the release of pro-inflammatory mediators (1). P2X7 has been implicated in a number of neurodegenerative diseases including amyotrophic lateral sclerosis (ALS). P2X7 is up-regulated in microglia of human ALS spinal cords (2) and rodents carrying G93A mutant superoxide dismutase 1 (mSOD1) (3,4).

Objectives: The aim of the current study was to determine whether murine EOC13 microglia express functional P2X7 receptors for use as a model to study the role of this receptor in ALS.

Methods: Murine J774 macrophages were used as a positive control for P2X7. P2X7 mRNA and protein expression were determined by RT-PCR and immunoblotting respectively. P2X7 function was measured using a fixed-time flow cytometric ethidium uptake assay.

Results: P2X7 mRNA and protein was present in murine EOC13 microglia. ATP induced ethidium uptake into EOC13 cells in a concentration-dependent manner and with an EC50 of 130 μM, typical of murine recombinant P2X7. The most potent P2X7 agonist, BzATP, also induced ethidium uptake. The P2X7 antagonists Brilliant Blue G,A438079,AZ10606120 and AZ11645373 impaired ATP-induced ethidium uptake by 90-100%.

Discussion and conclusions: These results demonstrate that EOC13 microglia express functional P2X7 receptors. Thus, this cell line may represent a potential *in vitro* model for further study into the potential roles of P2X7 in ALS. Studies investigating the up-regulation of P2X7 by mSOD1 in EOC microglia are planned.

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P131 THE ALTERNATIVE ACTIVATION OF MICROGLIA FROM ALS TRANSGENIC MICE IS ATTENUATED BY THE EXPRESSION OF MUTANT SOD1

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Keywords: microglia, mitochondria, PPAR

Background: Microglia are the resident macrophages in the central nervous system and screen their environment for insults. Activation of microglial cells is an early marker of disease in ALS and other neurodegenerative diseases. One activation status of microglia, the alternative activation, is characterized by a resolution of inflammation. We have recently shown that the alternative activation of microglia is inhibited by mitochondrial dysfunctions associated with neurodegenerative diseases, such as Parkinson's disease and Huntington's disease.

Objective: We hypothesize that mitochondrial dysfunctions in neurodegenerative diseases are directly linked to the inflammatory activation state of the microglia and that targeting the metabolic status of microglial cells might be a potential therapeutic approach for these diseases. We aimed to characterize the effect of the SOD1 mutation on the inflammatory profile of microglia and investigate potential pharmacological interventions.

Methods: We treated primary mouse microglia cells with agonists and antagonists of PPAR-receptors in combination with LPS and/or the alternative activation inducing cytokine IL-4 and quantified the production of IL-6. PPAR receptors play an essential part in the regulation of metabolism. In addition we isolated primary mouse microglial cells from the G93A SOD1 mouse model of ALS and investigated the alternative activation.

Results: We found that PPAR-alpha has a stimulatory effect on the alternative activation of microglial cells. We found that the alternative activation was reduced in microglial cells from transgenic mice with the mutated form of the SOD-1 gene in comparison to corresponding controls.

Conclusions: Targeting the metabolic system might therefore be a promising therapeutic approach for neurodegenerative diseases. This could be achieved by modulating the nature of the inflammatory response towards the alternative activation pathway.

P132 BCL2-A1 INTERACTS WITH PRO-CASPASE-3: IMPLICATIONS FOR AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: Bcl2-A1, pro-caspase-3, AP-1

Background: Expression of mutant SOD1 typical of ALS induces the expression of Bcl2-A1 (also known as Bcl2-related protein A1, BFL1; A1; Bfl-1/A1), a member of the Bcl2 family of proteins, specifically in motor neurons of transgenic mice already at the asymptomatic stage. Bcl2-A1 is protective against death of neuronal cells induced by expression of G93A-SOD1, but is detrimental upon stimulation of those cells with TNFalpha.

Objectives: We have investigated the molecular pathways leading to Bcl2-A1 transcriptional activation upon mutant SOD1 expression and the molecular mechanisms underlying the anti-apoptotic action of Bcl2-A1 in ALS cellular models.

Methods: Transfection of immortalized motor neurons (NSC-34), Western Blot GST-pulldown followed by Mass Spectrometry (MS) analysis and co-immununoprecipitation were used to study BCl2-A1 promoter and its interaction with pro-caspase-3.

Results: We report that up-regulation of Bcl2-A1 in ALS mouse model is limited to spinal cord and it is directly linked to mutant SOD1 expression, with a pattern that mirrors the tissue specificity of the disease. Although Bcl2-A1 is mainly expressed in lymphocytes, the present study indicates that constitutive mutant SOD1 overexpression does not modify either the expression level or the relative isoforms abundance in this cell type. Moreover, in immortalized motoneurons Bcl2-A1 is transcriptionally regulated by the redox sensitive transcription factor AP1, most likely contributing to lineage- and stimulus-dependent cell specificity of Bcl2-A1 transcription.

Using a GST-pull down approach combined to MS we were able to identify pro-caspase 3 as a binding partner for Bcl2-A1. This interaction is highly specific both *in vivo* and *in vitro* and it depends on Bcl2-A1 helix a9. Furthermore, Bcl2-A1 inhibits pro-caspase-3 activation in immortalized motor neurons expressing mutant SOD1 and thus induction of Bcl2-A1 in ALS mice represents a pro-survival strategy aimed at counteracting the toxic effects of mutant SOD1.

Discussion and conclusions: These data provide significant new insights on how molecular signalling, driven by expression of the ALS-causative gene SOD1, affects regulation of apoptosis in motor neurons. We also provide evidence of a new anti-apoptotic Bcl2-A1 mechanism of action. Bcl2-A1 can physically interact *in vitro* and *in vivo*, via helix a9, with pro-caspase-3, preventing its activation. To our knowledge, Bcl2-A1 is the only protein inhibitor of caspase-3 able to bind the zymogen precursor and to prevent its caspase-8 mediated activation, since both viral (serpin CrmA and p35) and cellular (IAP, inhibitor of apoptosis protein) inhibitors bind the activated form of this caspase.

P133 ULTRASTRUCTURAL STUDY OF SPINAL CORD MOTOR NEURONS IN ADAR2-DEFICIENT MICE

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Keywords: ADAR2-knockout mice, motor neuron, ultrastructure

Background: There have been some reports indicating that AMPA receptor-mediated excitotoxic mechanism plays a pathogenic role in ALS and SOD1-associated familial ALS model animals. Conditional ADAR2-knockout mice demonstrate that the loss of ADAR2 activity induces the slow death of motor neurons and are considered to be useful to research

on sporadic ALS. However, motor neurons of spinal cords have not been studied ultrastructurally in ADAR2-knockout mice.

Objectives: To examine ultrastructural alterations of motor neurons and to clarify whether the pathological changes are similar to those reported in sporadic ALS.

Methods: We electron-microscopically studied the motor neurons of cervical spinal cords in $ADAR2^{flox/flox}$ homozygous mice (15 weeks, n = 2), $ADAR2^{flox/+}$ heterozygous mice (74 wks., n = 2) and control mice (12 wks., 16 wks., 20 wks. and 24 wks., n = 2, respectively).

Results: Light-microscopically, on the plastic section of the cervical spinal cord of $ADAR2^{flox/flox}$ VAChT-Cre mice stained by toluidine blue, some large anterior horn neurons with simple atrophy were scattered in the anterior horn, accompanied by astrogliosis. Anterior horn neurons showed no vacuoles. There were no myelin ovoids in the white matter, anterior root or posterior root. In $ADAR2^{flox/+}$ VAChT-Cre mice, vacuoles of various sizes were observed in the cytoplasm and nuclei of posterior horn neurons as well as in anterior horn neurons. Myelin ovoids and the swelling of myelin were frequently found in the anterior column and, to a lesser extent, in the lateral and posterior columns, and anterior and posterior roots. Electron-microscopically, in $ADAR2^{flox/flox}$ VAChT-Cre mice, autophagosomes surrounded by a double-membrane and autolysosomes isolated by a single membrane were observed in the somata of anterior horn neurons. They contained sequestered cytoplasmic organelles such as mitochondria and ribosome-like structures. The cytoplasm and dendrites of motor neurons frequently contained electron-dense membranous structures. The cistern of ER was often dilated without accumulation of electron-dense material. Abnormal mitochondria containing electron-dense changes in the outer and inner membranes and cristae were frequently observed in the somata, dendrites and axons. In $ADAR2^{flox/+}$ VAChT-Cre mice, vacuolar changes were frequently demonstrated in the nuclei of motor neurons and astrocytes and, to a lesser extent, in the somata of motor neurons. The somata of anterior horn neurons often contained autophagy-related structures.

In controls, no vacuolar change was observed in the nuclei of motor neurons or astrocytes, while mitochondria occasionally showed electron-dense changes in the outer and inner membranes and cristae in the cytoplasm of motor neurons and the axons.

Conclusions: Pathological changes preferentially observed in the cytoplasm of spinal cord motor neurons in ADAR2-deficient mice are similar to those reported in sporadic ALS, but inclusions characteristic of ALS such as Bunina bodies, round bodies and skein-like inclusions have not been recognized in these mice.

P134 FUNCTIONAL ANALYSIS OF FUS/TLS MUTATIONS INVOLVED IN ALS

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Keywords: FUS/TLS, SMN, stress granules

Background: Onset of sporadic ALS before the age of 25 is rare, and has been hypothesised to constitute a distinct form of ALS on morphological grounds. We have recently reported a number of patients with a young onset form of ALS who presented with motor symptoms beginning between the age 17 and 22, rapid disease progression without dementia and no family history of ALS. At post-mortem, degenerating motor neurons showed intracellular basophilic inclusions staining positive for FUS protein and in three out of four of these patients, a mutation in the FUS gene was identified, in two cases a P525L change. The condition is therefore proposed to be a pathologically and genetically distinct form of sporadic ALS, juvenile ALS-FUS.

Objectives: The aim of this study was to provide *in vitro* correlation of the results obtained from post mortem tissue, looking for FUS co-localisation and interactions with RNA/DNA binding proteins involved in motor neuron disease.

Methods: HeLa cells and primary spinal motor neurons were transiently transfected with myc-tagged FUS containing the P525L or R521C mutation. Immunofluoresence was used to visualize the expression of FUS. Immunoprecipitation and Western blotting were performed to determine interactions of FUS.

Results: Immunofluorescence staining with confocal microscopy confirmed previous reports that WT FUS predominantly localizes to the nucleus whilst P525L-FUS, and to a lesser degree R521C-FUS, show a striking accumulation in the cytoplasm. Under conditions of oxidative stress, mutant FUS in the cytoplasm formed aggregates which also stained for the stress granule markers TiAL-1 and PABP. Double staining also revealed colocalization of mutant FUS and SMN, mutations in which cause spinal muscular atrophy. SMN from nuclear and cytoplasmic fractions was co-immunoprecipitated with anti-myc antibody indicating that SMN and myc-tagged FUS interact. The presence of mutations does not abrogate the SMN-FUS interaction.

Discussion and conclusions: In cells transfected with either the P525L or R521C FUS mutation we confirmed recent reports of mutant FUS cytoplasmic mislocalisation and accumulation in stress granules, suggesting that the insoluble inclusions seen at post-mortem originate from these stress granules. The transfected cells represent an *in vitro* system in which these preliminary results can be further investigated. Moreover we have shown, for the first time, that that there is co-localisation of SMN and FUS in these inclusions, and that SMN may interact with both WT and mutant FUS. Further investigation is required to explore the nature of the interaction of FUS and SMN within stress granules.

P135 STRESS KINASE ACTIVATION MODULATES ACCUMULATION OF TDP-43

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Keywords: TDP-43, JNK, metals

TDP-43 proteinopathies (FTLD-U and ALS) are characterized by loss of nuclear TDP-43 expression and C-terminal TDP-43 fragmentation and accumulation in the cytoplasm. Recent studies have shown that TDP-43 can accumulate in RNA stress granules (SGs) in response to cell stresses. This could be associated with subsequent formation of aggregates.

However, the pathway of endogenous TDP-43 accumulation in SGs during chronic disease is not understood. In this study we investigated the mechanism of TDP-43 processing and accumulation in SGs in neurons exposed to chronic oxidative stress. Neuronal and non-neuronal cultures were treated with the oxidative stress inducers paraquat or sodium arsenite and examined for TDP-43 and SG processing. We found that mild oxidative stress caused a loss of nuclear TDP-43, increased cytoplasmic accumulation of the 35 kDa C-terminal TDP-43 fragment and led to formation of TDP-43 and human antigen R (HuR)-positive SGs, a proportion of which were ubiquitinated. Our studies revealed that TDP-43 accumulation induced by chronic oxidative stress can form irreversible protein aggregates that remain present after removal of stress. In contrast, HuR rapidly dissociates from SGs upon removal of stress. The co-localization of TDP-43 with SGs could be blocked by inhibition of the stress kinase, c-Jun N-terminal kinase (JNK), without effect on the localization of HuR to SGs. In contrast, ERK or p38 inhibition prevented formation of both TDP-43 and HuR-positive SGs. The control of TDP-43 accumulation by kinases may be mediated through phophorylation of hnRNPs that co-localize with TDP-43 in SGs. To investigate therapeutic approaches to control TDP-43 accumulation, we co-treated neurons with a potentially therapeutic metallo-complex (CuII(atsm)). This was found to inhibit kinase activation and block TDP-43 accumulation. CuII(atsm) also increased expression of key cell survival proteins and reduced oxidative stress and neurotoxicity. In vivo studies revealed that CuII(atsm) inhibited abnormal TDP-43 processing, delayed disease onset and extended lifespan in G93A and G37R SOD1 murine models of ALS. Our studies are the first to demonstrate a critical role for kinase activation in TDP-43 accumulation and describe a potential therapeutic approach based on modulation of stress kinase activity. These findings may have important implications for development of treatments for FTD and ALS, targeting cell signal pathway control of TDP-43 aggregation.

P136 THE EFFECT OF EXTRACELLULAR STRESSES ON PATHOLOGY AND SURVIVAL/ DEATH REGULATION IN TDP-43-FALS LCLS

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Keywords: TDP-43, LCLs

Background: Transactive response DNA binding protein 43 kDa (TDP-43) is a major component of the ubiquitin-positive inclusions that are a pathological hallmark of affected cells in ALS. TDP-43 is an RNA/DNA binding protein that is predominantly nuclear but undergoes subcellular redistribution in ALS. It mislocalises to the cytoplasm and forms sequestered aggregates. Biochemical analyses of TDP-43 in brains and spinal cords of FTLD and ALS cases reveal that TDP-43 is pathologically modified. Since 2008, we and other groups have found missense mutations in the TARDBP gene encoding TDP-43 in familial and sporadic ALS cases, supporting a causative link between TDP-43 dysfunction and neurodegeneration.

Objectives: 1. Determine whether patient lymphocyte cell lines (LCLs) carrying ALS mutations can be used as a model for ALS; 2. To evaluate cell viability and proliferation activity of immortalized lymphocytes as a potential model of neuronal death in mutant TDP-43 familial ALS (TDP-43-FALS) cases.

Methods: Patient and control LCLs were treated with various stresses (apoptosis inducer; staurosporine, proteasome inhibitor; Mg132, and endoplasmic reticulum stress; thapsigargin) and were investigated using immunohistochemisty, immunofluoresence and Western blotting techniques. Growth curves of patient and control LCLs were obtained with trypan blue and MTS assay. Effect of extracellular stresses (staurosporine, Mg132, Thapsigargin) on patient and control LCLs was evaluated by using MTS and LDH assays.

Results: In treated LCLs carrying ALS mutations, TDP-43 was found to be pathologically modified: redistributed, ubiquinated, cleaved and hyperphosphorylated. There was no significant difference in the growth pattern and doubling time between patient and control LCLs. No difference in the endogenous level of apoptosis was found and also mutations did not seem to be rendering patient LCLs more susceptible to extracellular stresses.

Discussion and conclusion: Untreated lymphocytes did not show ALS pathology but lymphocytes treated with cellular stresses showed similar pathology to that of ALS patient cells, such as redistribution, aggregate formation, ubiquitination and phosphorylation, suggesting that these cells may be used to study disease mechanisms. The apoptotic pathophysiology in TDP-43-FALS was not reflected in the form of alteration in cell viability and proliferation activity under basal or stress stimulated conditions.

P137 THE RELATIONSHIP BETWEEN TDP-43 TRANSLOCATION AND MOTOR NEURON TOXICITY

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Keywords: TDP-43, apoptosis, caspase

Background: The disease mechanism underlying ALS is poorly understood. Increasing evidence suggests that apoptosis and mitochondria play key roles in the death of motor neurons associated with gene mutations. This motor neuron degeneration includes features that resemble apoptosis. DNA fragmentation and increased caspase 3 activity have been found in selective vulnerable central nerve system regions (1). It has also been suggested that SOD1 mutations lead to activation of several apoptotic regulators (2). However, the relationship between mutant TDP-43 and neuro-toxicity remains poorly understood. Better knowledge of the death mechanism will also facilitate development of potential therapeutic targets.

Objective: 1) To identify the death signalling pathway of mutant TDP-43 induced toxicity; 2) To study the relationship between TDP-43 mislocation and neuron-toxicity.

Methods: Transfected NSC34 cells or fibroblasts were treated with a pan-caspase inhibitor Z-DEVD and/or a proteasome inhibitor MG132. MTT assay and LDH assay were performed to determine cell toxicity. Cells were visualised using confocal microscopy and the cells with TDP-43 mislocation

were counted. Western blotting was performed to study the activation of caspase 3. JC-1 assay was used to study the effect of mutant TDP-43 on mitochondria.

Results: In our experimental systems, both NSC34 cells and stressed fibroblasts showed TDP-43 translocation from nucleus to cytoplasm. Overexpression of mutant TDP-43 caused a 4-fold increase in cells with cytoplasmic TDP-43 and caused a higher toxicity (68% ± 3.6 viability in mutant vs 100% ± 6.2 in control). We found TDP-43 cytoplasmic inclusions in mutant fibroblast cells, but not in control cell, even before treatment. The mutation carrying fibroblast cells also had a higher percentage of TDP-43 mislocation (34% ± 0.4, compared to control 13% ± 0.4) and showed a higher toxicity by LDH assay after treatment. We observed caspase 3 activation and mitochondrial membrane permeabilisation in transfected NSC34 cells. The level of activated caspase 3 was higher in mutant TDP-43 transfected cells than wild type. In an attempt to reduce the toxicity, a pan-caspase inhibitor Z-DVED was used. However, this was unable to reduce cytotoxicity in either wild type or mutant TDP-43 expressing NSC34 cells, regardless of the presence or absence of MG132.

Discussion and conclusions: In this study, we found that mutant TDP-43 caused greater cytotoxicity than overexpressing wild type TDP-43. TDP-43 mutation also rendered fibroblasts more susceptible to cellular stresses. Both caspase 3 and mitochondria were involved in TDP-43 mediated cytotoxicity, suggesting this toxicity was due to apoptotic mechanisms. There was a difference in caspase 3 activation between wild type and mutant TDP-43 transfected cells, suggesting cells carrying TDP-43 mutations may be more likely to undergo apoptosis. The inefficiency of a pan-caspase inhibitor suggested that the toxicity induced by TDP-43 may be regulated by a range of molecules, not only caspases. Work is currently underway to better understand these mechanisms.

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P138 EXPRESSION OF MUTANT TDP-43 ENHANCES CELLULAR SUSCEPTIBILITY TO IMPAIRED ENERGY METABOLISM

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Keywords: TDP-43, energy metabolism, cell culture model

Background: The TAR DNA-binding protein (TPD-43) is a common component of cytoplasmic inclusions present in the majority of ALS cases and other neurodegenerative diseases including frontotemporal lobar dementia with ubiquitinated inclusions, Alzheimer's disease and dementia with Lewy bodies. The fundamental causes of aberrant TDP-43 metabolism and cellular localisation are not completely understood but the association of TDP-43 mutations with some forms of ALS indicate mutations to TDP-43 induce and/or accelerate neuronal dysfunction and degeneration.

Objectives: The objective of this study was to directly compare cells expressing normal or mutated TDP-43 and to examine how mutations to TDP-43 affect cell growth and TDP-43 metabolism.

Methods: NSC-34 cells were transfected to express non-mutated human TDP-43 (WT-TDP-43) or human TDP-43 harbouring the A315T mutation present in familial forms of ALS (A315T-TDP-43). Growth of the cells was measured in real time over several days using the xCELLigence cell analyser. As a model to examine the effects of impaired energy metabolism, the cells were grown in normal media with adequate glucose levels or in low glucose media where glucose levels become limiting. Cellular distribution of TDP-43 was determined by fluorescence microscopy and expression of TDP-43 and other cellular proteins determined by Western blot.

Results: Real time cell growth analysis revealed the expression of A315T-TDP-43 impairs growth of NSC-34 cells under normal growing conditions. Further to this, decreasing the availability of glucose in the culture media demonstrated that while both cell lines were sensitive to conditions that limit cellular energy metabolism, these sub-optimal growing conditions have a greater impact on cells expressing the A315T TDP-43 mutation. After 6 days in culture under normal growing conditions the cellular location of both WT-TDP-43 and A315T-TDP-43 is almost exclusively nuclear. Under conditions of low glucose however the A315T-TDP-43 is predominantly cytoplasmic, compared to the WT-TDP-43 which remains nuclear. Western analyses revealed the low glucose growing conditions did not significantly alter overall expression levels of WT- or A315T-TDP-43. By contrast, expression of the survival of motor neuron protein (SMN) was significantly decreased under conditions of limited glucose availability, but only in cells expressing the A315T-TDP-43.

Discussion and conclusions: These cell culture studies indicate that motor neurons expressing mutated forms of TDP-43 are more susceptible to conditions in which energy metabolism is impaired. Under these conditions mutated TDP-43, but not WT-TDP-43, translocates to the cytoplasm. This abnormal redistribution of TDP-43 may contribute to the decline in motor neuron function and viability by disrupting homeostasis of proteins such as SMN.

P139 FAMILIAL ALS-LINKED TDP-43 MUTATIONS CAUSE THEIR PROTEIN STABILIZATION AND DYSREGULATE MRNA METABOLISM

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Keywords: TDP-43, protein stability, mRNA stability

Background and objective: Abnormal accumulation of TDP-43 was identified as a pathological hallmark of ALS and FTLD. More than 30 missense mutations in TDP-43 gene, most of which reside at C-terminus of the protein, have been identified in both sporadic and familial ALS patients. Hyperphosphorylated 25 kDa C-terminus TDP-43 fragments are accumulated and aggregated in the motor neurons of ALS patients (1, 2). Since C-terminal fragments actually behave as seeds to facilitate cytosolic aggregation of full-length TDP-43 in the culture cell model, these fragments may be relevant to motor neuron degeneration in ALS (3). However, the detailed

cytotoxic mechanisms of ALS-linked TDP-43 mutant protein remain to be unclear. Therefore, the aim of our study is to elucidate the cytotoxic mechanisms of mutant TDP-43 by biochemical approaches.

Methods: Our experiments were carried out using mouse neuroblastoma Neuro2a cells as an *in vitro* model. We constructed a non-tagged TDP-43 expressing vector in order to eliminate the effect of artificial tag. First, wild-type and all familial ALS-linked (fALS) mutant TDP-43 proteins were transiently expressed in differentiated Neuro2a cells with dibutyl cyclic AMP, the localization and solubility of TDP-43 proteins were analyzed by differential centrifugation and solubilization, respectively. Next, to measure the stability of TDP-43 proteins in cells, half-lives of several fALS-linked mutant TDP-43 proteins were determined by pulse-chase experiment. To measure the exon-skipping activity and the mRNA destabilization activity, the monitoring plasmids containing CFTR exon 9 mini-gene and GFP fused TDP-43 3'UTR were constructed, respectively. The activity of TDP-43 was determined by co-transfection with these monitoring vectors.

Results and discussion: The localization and solubility of TDP-43 were not altered by the introduction of mutation under normal conditions. Mutant TDP-43 proteins, however, had longer half-lives than the wild-type. Intriguingly, half-lives of mutant TDP-43 proteins were negatively correlated with the onset age of ALS, but not with the duration. In addition, the mRNA destabilization activity of fALS-linked mutant TDP-43 proteins was approximately 6 fold lower than that of wild-type in the differentiated neuroblastoma cells. On the other hand, the exon-skipping activities were not lost in the mutant TDP-43 proteins.

Among these results, significant differences have been observed in both the protein stability and the mRNA destabilization activity. We therefore speculate that they are the key phenomena to understand the cytotoxic mechanism of mutant TDP-43 proteins. We are in process of investigating why fALS-linked mutant TDP-43 proteins impair mRNA regulation through protein stabilization. The detailed results will be reported and discussed.

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P140 MUTANT TDP-43 AND ASTROCYTES

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Keywords: astrocytes, GTPases, TDP-43

Background: The RNA-binding protein TDP-43 has recently been demonstrated as an aetiological factor in motor neuron disease (MND). TDP-43 is a DNA and RNA binding protein regulating transcription and splicing. TDP-43 is also involved in transport and local post-transcriptional modification of mRNAs. The protein is abundantly expressed in motor neurons and astrocytes. TDP-43 pathology is triggered by abnormal processing and cytosolic aggregation of the protein or by mutations in the TDP-43 gene. The pathology is similar and the outcome is directly linked to cell death. Mutant TDP-43 causes familial forms of human MND, MND-like disease in transgenic animals and kills motor neurons in primary culture.

TDP-43 pathology is also found in astrocytes: a cell type that plays critical roles in the pathology of MND. Astrocytes are the major contributor to defence against oxidative damage and express transporters that clear excess glutamate from the extracellular space. Loss of these transporters leads to excitotoxic damage. In MND, changes in astrocyte physiology occur prior to those of motor neurons and their severity can be correlated to disease progression. Thus, the extent of preservation of key astrocytic properties and the ability of these cells to mount appropriate defensive responses are important determinants of tissue viability in many neurological diseases.

Methods: We have established cellular models of TDP-43 proteinopathies by expressing fluorescently-tagged TDP-43 (wild-type and mutants) in astrocytes in primary cultures. We have also silenced TDP-43 expression in these cells. We have used these models to investigate the role of TDP-43 and its mutants on normal cell function and on the response of these cells to injury.

Results: Presence of TDP-43 mutations, caused reorganisation of the actin cytoskeleton and lead to impaired wound healing in an in vitro injury model ($n = 5$). Moreover, astrocytes transfected with the Q133k TDP-43 mutation had decreased expression of GLT-1 glutamate transporters ($n = 3$) and displayed impaired mitochondrial function as evaluated by the mitochondrial membrane potential ($n = 3$). These cells also had an impaired ability to withstand oxidative stress ($n = 3$). Finally, the presence of mutant TDP-43 increased the activists of the Rho family GTPases Rho A and Rac-1 while significantly reduced Cdc42 activity suggesting a direct role for TDP-43 in the regulation of the Rho-family GTPases.

Discussion and conclusions: These results demonstrate severe effects on normal astrocytic function by mutant TDP-43 and suggest that astrocytes could play a role in motor neuron demise in TDP-43 proteinopathies.

P141 CONTACT WITH AGGREGATED SOD1 CAUSES EFFICIENT ACTIVATION OF MICROGLIA AND ASTROCYTES *IN VITRO*

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Keywords: microglia, astrocytes, SOD1

Background: A large body of literature suggests that amyotrophic lateral sclerosis pathology is intimately linked with neuroinflammation, specifically activation and recruitment of microglia and astrocytes. The actual cause of gliosis is unclear. However, extracellular Cu/Zn superoxide dismutase (SOD1) has recently been shown to activate microglia providing one potential mechanism by which glial cells activated (1).

Objectives: As protein inclusions are thought to be an important part of ALS pathology and are associated with all forms of ALS we sought to determine if aggregated SOD1 would activate microglia and/or astrocytes.

Methods: Recombinant SOD1 was expressed in *E.coli* in conjunction with its copper chaperone to ensure correct metal

loading. The resulting enzyme showed correct secondary structure and enzyme activity. To promote aggregation, SOD1 and a small panel of mutants were incubated with 30 mM Dithiothreitol (DTT) and 5 mM Ethylenediaminetetraacetic acid (EDTA), (to break disulphides and remove metals respectively). The samples were extensively dialyzed against PBS after aggregation to remove DTT and EDTA. Aggregated and monomeric forms of SOD1 were then added to either microglia or astrocyte cells in culture. To ensure that the effects we observed were not due to LPS contamination we also used the EOC-13 microglial cell line (LPS non responsive) and or compared the response to treating the cells with LPS. Cell lysates were collected for Western blot analysis and supernatants were analysed for cytokine secretion via ELISA kits.

Results: Both monomeric and aggregated SOD1 bound to the surface of glial cells and was internalized. Although monomeric mSOD1 has been shown to promote microglial activation in the past we found that aggregated SOD1 was able to much more efficiently activate microglia and astrocytes in culture when compared to the monomeric form of mSOD1.

Conclusion: We have for the first time shown that aggregated mSOD1 potently activates both microglia and astrocytes. These results suggest that aggregated SOD1 is potentially more neurotoxic than monomeric SOD1 and may play a key role in disease progression in ALS.

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P142 ROLE OF EXTRACELLULAR SOD1 IN THE PATHOGENESIS OF ALS

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Keywords: extracellular SOD1, uptake, ER stress

Background: Mutations in the superoxide dismutase 1 (SOD1) gene account for about 20% of familial ALS and the presence of SOD1 inclusions is also a common pathological feature in sporadic ALS. SOD1 has been previously shown to be secreted by neuronal cell lines and is found in the CSF of familial and sporadic ALS patients. The exact role of this extracellular SOD1 remains unclear. This study shows that extracellular SOD1 is taken up by motor neuron like NSC34 cells and the uptake of mutant SOD1 results in the activation of ER stress. Our laboratory has shown that activation of ER stress occurs early in the pathogenesis of both familial and sporadic ALS. Thus the uptake of extracellular SOD1 could be a possible mechanism for the spread of neurotoxicity among motor neurons in ALS.

Objectives: The aims of this study were to investigate whether motor neurons could uptake extracellular SOD1, and whether the uptake of SOD1 could induce ER stress in these neurons.

Methods: Motor neuron-like NSC-34 cell lines were cultured and treated with recombinant SOD1 protein. Immunocytochemistry, epifluorescence microscopy and immunoblotting were performed to detect SOD1 uptake in these cells. The cells were also examined for ER stress markers.

Results: Immunocytochemistry and immunoblotting results showed that extracellular SOD1 is taken up by NSC-34 cells and the uptake of mutant G93A SOD1 resulted in marked increase of ER stress markers such as CHOP. The addition of mutant SOD1 also induced morphological changes indicative of cell death in NSC-34 cells.

Discussion: The mechanism of neurodegeneration in ALS is non-cell autonomous, a process by which an affected cell (motor neurons or glial cells) induces the disease phenotype in other neighbouring cells. This study has shown the uptake of extracellular SOD1 by motor neuron NSC-34 cells, indicating that cell to cell transmission of SOD1 could be involved in ALS. Hence SOD1 could be a possible toxic factor responsible for the non-cell autonomous mechanism in ALS. These results also suggest that targeting the pathways activated by extracellular mutant SOD1 could be a possible therapeutic approach for ALS.

P143 EXOGENOUS MUTANT SOD1 CAN BE INTERNALIZED BY MOTOR NEURONAL CELLS AND PROMOTES FORMATION OF INTRACELLULAR PROTEIN INCLUSIONS

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Keywords: protein aggregation, NSC-34, green fluorescent protein

Background: Clinical evidence shows that motor neurone degeneration in Amyotrophic Lateral Sclerosis (ALS) is a process which starts focally and spreads progressively through the nervous system (1). Like many other neurodegenerative diseases, protein inclusions are closely linked to ALS pathology. Inclusions positive for Cu/Zn superoxide dismutase 1 (SOD1) have been found in familial ALS with mutations in SOD1, and studies have also found inclusions of misfolded wild-type SOD1 in sporadic ALS patients (2). Recent evidence suggests that a transfer of SOD1 aggregates emulate a prion-like mechanism (3). Collectively, these data suggest that aggregate propagation may play a role in ALS pathology.

Objectives: The aim of this study is to demonstrate whether exogenously applied aggregates and monomeric mutant or wild-type SOD1 can seed the formation of intracellular inclusions in both wild-type or mutant SOD1 expressing cells.

Methods: Recombinant mutant or wild-type SOD1 was co-expressed with the copper chaperone to ensure correct metal loading in E.coli, then purified through size-exclusion and anion exchange chromatography. Recombinant SOD1 was incubated with 50 mM dithiothreitol (DTT) and 5 mM ethylenediaminetetraacetic acid (EDTA) for 70 hours with agitation to promote aggregation. The samples were then dialysed in Phosphate buffer saline to remove DTT and EDTA. NSC-34 cells were transiently transfected with human SOD1-EGFP-SOD1 expression constructs (wild-type, G93A, or EGFP alone). Aggregated and monomeric G93A or wild-type recombinant protein was added on to culture of transfected NSC-34 cells. The proteasome inhibitor MG132 acted as a positive control for inclusion formation promotion. At early time points, cell lysates were collected for Western blot analysis while at later time points cells were analysed by confocal microscopy.

Results: Western blot analysis demonstrated insoluble protein aggregates were taken up by NSC-34 cells, this occurred

as early as 2 h. Confocal imaging showed that protein inclusions were promoted by incubation with either monomeric or aggregated mutant SOD1. This was the case in both WTSOD1-EGFP and G93ASOD1-EGFP expressing cells.

Discussion and conclusions: This study suggests that exogenous SOD1 aggregates can be internalized by motor neurone like cells and initiate the formation of intracellular protein inclusions. We propose that these inclusions are toxic to cells leading eventually to further release of aggregated proteins and subsequently take up by neighbouring cells. This mechanism may explain the progressive pathology of ALS.

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P144 A NOVEL MECHANISM OF SET/I2PP2A SHUTTLE BETWEEN NUCLEUS AND CYTOPLASM

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Keywords: Alzheimer's Disease, SET/I2PP2A, nuclear localization signal (NLS)

Background and objectives: SET/I_2^{PP2A} acts as a key impetus on neurodegenerative disease, especially Alzheimer's Disease (AD) onset. Until now, little has been known about the detailed regulatory mechanism by which SET was detained in cytoplasm and the consequent events in mammalian cells. Phosphorylation is the best-understood mechanism of regulation of nuclear transport. SET sequence (6-11aa) AKVSKK almost coincided with the consensus nuclear localization signal (NLS) sequence KKXXKX or XKXXKK. Therefore, the present study is to explore whether the sequence ^{6}AKVSKK^{11} of SET is a potential NLS, and plays an essential role on SET nuclear import.

Methods: Site-directed mutagenesis; HEK293/tau cells culture and transfection; confocal microscopy; cytoplasmic and nuclear extraction; Western blot; immune-precipitation.

Results: 1) Pseudophosphorylation of SET at serine 9 was observed mostly in cytoplasm, while nuclear accumulation of pseudounphosphorylated SET and wt SET was not significantly affected. 2) Phosphorylation of SET at Serine 9 significantly reduced SET/karyopherin complex formation compared to npSET and wtSET. 3) Lysine11 mutation to alanine induced SET exclusively localizing in cytoplasm while K7A and K10A SET still mainly stayed in nucleus. 4) PP2A activities in either pSET or wtSET in treated HEK293/tau cells were significantly decreased as compared with that of control group, but npSET didn't influence PP2A activity. Interestingly, when directly comparing between pSET and wtSET, we also found that pSET inhibited PP2A activity more efficiently than wtSET by 25.2% ($p = 0.006$).

Conclusion: Phosphorylation of SET at Ser 9 inhibits SET nuclear import and promotes an inhibition of PP2A activity.

P145 ENDOCYTIC TRANSPORT ABNORMALITIES AS A DETERMINANT OF MOTOR NEURON VULNERABILITY IN ALS

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Keywords: endosome, exosome, Rab

Background: ALS is characterised by accumulation of pathological misfolded proteins in vulnerable motor neurons by unclear mechanisms. Increasing evidence suggests a key role for membrane trafficking defects in ALS neurodegeneration. Many ALS genes encode molecular machinery or enzymes directly mediating intracellular transport; these gene products are often cargoes of transport vesicles, and defective transport features early and prominently in ALS models. The endosome-lysosome system (ELS) is a major intersection for intracellular traffic and responsible for autophagic clearance and exosomal secretion of misfolded proteins. Both autophagic activation and exosome abnormalities are reported in ALS models, suggesting a disruption to the ELS.

Objectives: We hypothesised that abnormal endosome transport is a common pathogenic process engaged by leading ALS-linked proteins such as SOD1, TDP-43 and FUS. ELS morphology and transport was therefore examined in ALS models and patients.

Methods: NSC-34 cells stably expressing normal or mutant SOD1, TDP-43 and FUS were studied for endocytic Rab and autophagic markers by Western blotting and immunocytochemistry. Cells were treated with endocytic tracers including fluorophore-tagged transferrin and dextran to measure endosome transport rate. Cells were separately co-transfected with endocytic Rab-GFP or ubiquitin-RFP to modulate endosome transport. Lastly, endosome pathology was examined in post-mortem spinal cord tissues from sporadic ALS patients ($n = 10$) and non-neurological disease controls ($n = 5$).

Results: We first demonstrated that SOD1, TDP-43 and FUS were secreted by cell-derived exosomes and that ALS-linked forms of these proteins were depleted in exosomes, preceding cytoplasmic inclusion formation, ER stress and cell death activation. Exosome deficits correlated with early endosome defects determined by abnormal Rab5 upregulation, enlarged early endosomes and endocytosis of tracers. Rab5 induction was confirmed in spinal cords of presymptomatic transgenic $SOD1^{G93A}$ mice and sporadic ALS patients, unlike controls.

Discussion and conclusions: Based on these findings, we propose that early endosome transport defects leading to impaired exosomal secretion and increased cytoplasmic protein burden may be an early determinant of motor neuron loss and common denominator of key pathological ALS-linked proteins. Abnormal endocytic transport is likely to point to more fundamental mechanisms of vesicle trafficking defects implicated in ALS and innovative potential therapeutic approaches.

P146 DEFECTIVE RELOCALIZATION OF ALS2/ALSIN TO RAC1-INDUCED MACROPINOSOMES ACCOUNTS FOR LOSS OF THEIR CELLULAR FUNCTION AND LEADS TO DISTURBED AMPHISOME FORMATION

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Keywords: ALS2/Alsin, autophagy, amphisome

Background: ALS2, the causative gene product for juvenile recessive motor neuron diseases, regulates macropinocytosis and following endosome fusion. Recently, we reported that loss of ALS2 in SOD1^{H46R} mice resulted in an earlier death and accelerated accumulation of abnormal autophagosomes in the spinal axons. These findings suggest that ALS2 acts as a positive regulator for autophagolysosome-mediated protein degradation, and loss of its function leads to malfunction of autophagolysosome-mediated protein degradation in neurons. Consistently, growing evidence supports the notion that impairment of autophagy (autophagosome formation, maturation and degradation) is indeed associated with the formation of protein aggregates and neurodegeneration. Thus, autophagy serves an adaptive role to protect neurons from degeneration. However, the molecular mechanisms by which loss of ALS2 results in the accelerated accumulation of abnormal autophagosomes remain elusive.

Objectives: To clarify the molecular basis for phenotypic modification of autophagosome trafficking and maturation by loss of the ALS2 function.

Methods: We investigated Rac1-induced relocalization of ALS2^{WT} or its pathogenic mutants (ALS2^{C157Y} and ALS2^{G540E}) using HeLa cells. We also performed indirect immunofluorescent analysis to observe the colocalization of ALS2 with EEA1 (endosome marker) and microtubule-associated protein 1A/1B-light chain 3 (LC3) (autophagosome marker). The association of ALS2 with specific lipid molecules was examined by PIPStrip overlay assay using purified FLAG-ALS2.

Result: First, we observed the localization of ALS2^{WT} and its mutants in Rac1^{Q61L} expressing cells. Interestingly, unlike ALS2^{WT}, both ALS2^{C157Y} and ALS2^{G540E} failed to be localized to both Rac1^{Q61L}-induced macropinosomes and macropinosome-derived early endosomes (EEs), and yet sequestered in the cytoplasm. Next, to clarify the mechanisms for the mislocalization of the ALS2 mutants, we examined whether the ALS2 mutants could directly bind to phosphoinositide phosphates (PIPs), membrane-compartment specific lipid molecules. Both FLAG-ALS2^{C157Y} and FLAG-ALS2^{G540E} exhibited lower affinities to PI(3)P and PI(4)P compared to FLAG-ALS2^{WT}. Lastly, we investigated the effect of ALS2 mislocalization in autophagosome trafficking and maturation. Expression of ALS2^{WT} induced enlarged EEA1-positive vesicles that were colocalized with LC3, indicating an enhanced formation of amphisomes. Notably, expression of neither ALS2^{C157Y} nor ALS2^{G540E} enhanced the formation and/or enlargement of amphisomes.

Discussion and conclusions: We showed that loss of the affinities to specific lipid molecules accounted for the mislocalization of pathogenic ALS2 mutants in cells. Since defective relocalization of ALS2 leads to loss of the ALS2 function as a Rab5 activator on macropinosomes/endosomes, resulting in disturbance of the autophagosome/endosome maturation, malformation of amphisomes might underlie the pathogenesis of the *ALS2*-linked MNDs. Future studies on the molecular basis of ALS2 will uncover the roles of macropinocytosis and amphisome formation in selective cargo sorting and degradation, which brings new insights into the pathogenesis for *ALS2*-linked MNDs and other neurodegenerative diseases.

P147 DISTINCTIVE HIGH-MOLECULAR WEIGHT OLIGOMERIC COMPLEXES OF ALS2/ALSIN ARE ENRICHED IN THE BRAIN SYNAPTOSOMAL COMPARTMENTS

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Keywords: ALS2, synaptosome, protein complex

Background: The loss-of-functional mutations in the *ALS2* gene account for a number of recessive motor neuron diseases (MNDs). Thus, the *ALS2* gene product, ALS2/alsin, plays an important role in maintenance and/or survival of motor neurons. Previously, we have demonstrated that ALS2 acts as a guanine nucleotide exchange factor (GEF) for a small GTPase Rab5, and forms a homophilic oligomer. This homo-oligomerization is crucial for the Rab5-GEF activity *in vitro* and for the ALS2-mediated endosome enlargement in cultured cells. Moreover, the intracellular distribution of ALS2 is drastically shifted from cytosol to the membrane/vesicle compartments by the activation of an ALS2-upstream factor Rac1, thereby inducing endosome enlargement in its Rab5-GEF activity-dependent manner. These findings prompted us to hypothesize that the conformational changes and/or differences in the ALS2 complex might be associated not only with its intracellular distribution but also with the tissue-specific ALS2 function *in vivo*. However, it remains unclear whether there are differences in the conformation of the ALS2 complex in different cellular compartments among different tissues, let alone whether the conformation-activity relationship of the ALS2 complex is implicated in its physiological function.

Objectives: To delineate the conformation-activity relationship of the ALS2 complex *in vivo*.

Methods: Whole brain tissue from C57BL/6N mouse was lysed, and subjected to immunoprecipitation using the anti-ALS2 antibody, followed by MALDI-TOF/MS, to detect possible ALS2 interactors *in vivo*. Whole brain and liver from mice were homogenized and fractionated by a series of centrifugations, resulting in subcellular fractions; P1, P2, P3, and S3. Synaptosomes were enriched from P2 by the gradient-centrifugation, and soluble synaptosomal fraction (P2-S) was obtained from synaptosomes by the treatment with non-ionic detergent. P2-S and S3 fractions were subjected to gel-filtration analysis with the use of the Superose 6 HR 10/30 column. By Western blot analysis, the subcellular distribution of ALS2 and molecular weight of the ALS2 complex were determined.

Results: In the brain, no major ALS2 interactors other than ALS2 itself were detected. In the liver, ALS2 was almost

exclusively distributed in S3 (cytosol). By contrast, ALS2 was mostly enriched in P2 in the brain. Gel-filtration analysis revealed that cytosolic (S3) ALS2 from either the liver or brain tissue was eluted at an apparent peak molecular masses of ~750 kDa, indicating that ALS2 forms a tetramer. Remarkably, ALS2 in P2-S showed a distinctive high-molecular weight distribution (presumably octamer) in addition to their tetrameric complex.

Discussion and conclusions: Our interim results suggest that the subcellular distributions of ALS2 in the CNS and peripheral tissues are different, and that the synaptosomal high-molecular weight ALS2 complex may be associated with neuron-specific ALS2 function *in vivo*. Currently, to delineate the conformation-activity relationship of the ALS2 complex, biochemical analyses of the wild-type and mutant ALS2 complexes are underway.

P148 THE FAILURE OF ER-GOLGI TRANSPORT IN MOTOR NEURON-LIKE CELLS EXPRESSING MUTANT SOD1 PROTEINS

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Keywords: SOD1, ER-Golgi transport, VSVG

Background: Increased evidence shows that axonal transport and endoplasmic reticulum (ER) stress are the pathogenic mechanisms of ALS. We previously showed that ER stress is triggered early in the disease process, suggesting a significant role in pathophysiology. Motor neurons are large cells with long axons and are particularly susceptible to the failure of axonal and cellular transport. However, how ER stress is triggered in cells expressing mutant SOD1 proteins has not been established. Transfection of a gene encoding an abundant membrane glycoprotein (G protein) from vesicular stomatitis virus (VSV) in culture cells rapidly synthesize the VSVG protein on the ER like normal secretory protein. Use of mutant encoding a temperature sensitive VSVG (VSVGtsO45) allows the subsequent transport of this protein from ER to Golgi.

Objective: In this study, we investigated whether the transport of VSVGtsO45 from ER to Golgi is inhibited in cells expressing mutant SOD1 proteins and whether the failure of ER-Golgi transport occurs before ER stress in cells expressing mutant SOD1. Furthermore, we also examined whether overexpression of Sar1 (a COPII subunit) or protein disulphide isomerase (PDI) rescues ER-Golgi transport in cells expressing mutant SOD1.

Methods: NSC-34 cells were co-transfected with SOD1-EGFP vectors (either WT or mutants) and VSVGtsO45-mCherry. For overexpression of Sar1 or PDI, cells were triple transfected with SOD1-EGFP vectors (either WT or mutants), VSVGtsO45-mCherry and Sar1-Flag or pCMV-PDI vectors. After transfection, cells were then stained with ER or Golgi markers to determine whether VSVGtsO45 proteins are localized in the ER or Golgi.

Results: After 72 h transfection, the inhibition of VSVGtsO45 transport from ER to Golgi is significant in cells expressing mutant SOD1 proteins. This inhibition begins at 16 h transfection while ER stress occurs at 18 h transfection, and inclusions are only seen at 24 h transfection time points. Overexpression of Sar1 or PDI rescues transport of VSVGtsO45 from ER to Golgi in cells expressing mutant SOD1 proteins. In addition, mutant TDP-43 proteins also block the ER-Golgi transport of VSVGtsO45 in NSC-34 cells.

Discussion and conclusion: These data provide the evidence that ER-Golgi transport of proteins is restrained in cells expressing mutant SOD1 proteins. The failure of ER-Golgi transport occurs early and before ER stress, indicating that it triggers ER stress in cells expressing mutant proteins. Overexpression of Sar1 or PDI rescues ER-Golgi transport, suggesting a possible neuroprotective role in motor neuron death. Mutant TDP-43 may act similarly to mutant SOD1 in ALS pathogenesis.

P149 G93A HSOD1 IMPAIRS MITOCHONDRIAL CALCIUM HANDLING AND CAUSES ER STRESS IN EMBRYONIC MOTOR NEURONE CULTURES

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Keywords: mitochondrial dysfunction, calcium dynamics, UPR

Background: Motor neurones vulnerable to amyotrophic lateral sclerosis (ALS) express low amounts of calcium binding proteins (1,2) so intracellular calcium passing through the ER mitochondria calcium cycle (ERMCC) must be buffered primarily in mitochondria (3). Isolated G93A hSOD1 mitochondria show reduced calcium buffering capacity (4). FCCP releases less calcium from G93A hSOD1 mitochondria (5,6), but studies on ERMCC dynamics in mutant hSOD1 motor neurones with near physiological stimuli are missing.

Objectives: To analyse cytosolic and mitochondrial calcium transients after very brief AMPA receptor activation comparing non-transgenic and G93A hSOD1 motor neurones. Further to evaluate if impaired mitochondrial calcium buffering leads to ER stress and activation of the unfolded protein response (UPR).

Methods: Mixed motor neurone cultures were prepared from E13 ventral spinal cord of non-transgenic and G93A hSOD1 mice. AMPAR were stimulated with kainate for 2 s. Cytosolic calcium transients were measured using fura-2 AM and mitochondrial calcium dynamics with rhod-2 AM. CGP 37157 was applied to inhibit mitochondrial calcium export through the mitochondrial Na/Ca exchanger thereby reducing the calcium shuttling back to the ER. Markers of the unfolded protein response were visualised using immunofluorescence.

Results: Non-transgenic motor neurones ($n = 18$) had a slightly faster decay of cytosolic calcium transients than non-motor neurones ($n = 18$). Calcium decay was decelerated in G93A hSOD1 motor neurones ($n = 12$) compared to the non-transgenic motor neurones. Whereas G93A hSOD1 non-motor neurones ($n = 17$) showed a cytosolic calcium decay comparable to the non-transgenic non-motor neurones. Mitochondrial calcium export was accelerated in G93A hSOD1 motor neurones compared to non-transgenic motor neurones, possible causing higher cytosolic calcium levels. The treatment with CGP 37157 activated the UPR in non transgenic motor neurones, demonstrating that reduced mitochondrial calcium release can introduce ER stress.

Discussion and conclusions: The results demonstrate that G93A hSOD1 induces disturbance of the cytosolic calcium decay after a near physiological activation of AMPA receptors. This is caused by a reduced calcium retention capacity in mitochondria of G93A hSOD1 motor neurones, and triggers ER stress. The study provides evidence that mitochondrial calcium handling is crucial for maintaining ER function, and that motor neurones are particularly vulnerable to ERMCC dysfunction. EMRCC stabilization may therefore provide a new therapeutic principle in motor neurone disease.

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P150 MODELING RETROGRADE DEGENERATION OF MOTOR NEURON AXONS AS A CONSEQUENCE OF MITOCHONDRIAL DYSFUNCTION

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Keywords: energy metabolism, electrophysiology, computer model

Background: Mitochondrial dysfunction is now recognized as a fundamental player in many neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). In ALS, anomalous mitochondria are found early in the disease in humans and in the SOD1 mouse model. The role of mitochondrial dysfunction in triggering the selective degeneration of fast motorneurons is still unknown since mitochondria have many functions. However, one of the most essential is to produce the ATP needed for homeostatic processes such as pumping ions across the cell membrane and so we decided to model the consequences of reduced ATP for motorneuron function and survival.

Methods: To better assess the consequences of mitochondrial perturbations and specifically of reduced ATP production, we built a realistic computer model of a spinal motorneuron. This model is based on motor neuron morphology, reproduces realistic firing properties and includes biochemical pathways involved in the production, distribution and consumption of ATP. The model emphasizes the role of ATP in running Na + /K + pumps and maintaining motorneuron homeostasis. We use this model to study the requirements for ATP production and distribution in maintaining the electrical properties of the neuron and holding the intracellular calcium concentration in a normal range.

Our results show: 1) When ATP levels reach a critical lower bound, an electrical instability develops, leading to a maintained depolarized state, and causing massive calcium influx. The instability leads to high energy consumption, further worsening the energy deficit. This pathological state is not spontaneously reversible and is likely to lead to neuronal degeneration and death. 2) If the ATP deficit is localized to the distal end of the axon, the instability depolarizes the terminals, triggering ectopic action potentials reminiscent of the hyperexcitability associated with fasciculation potentials. More importantly, this instability can spread in a retrograde fashion until it reaches the soma. 3) Finally, the vulnerability of each neuron to this instability strongly depends on its specific morphology and firing properties. We tested the hypothesis that selective motor neuron vulnerability in ALS might be linked to specific firing and energy profiles. We find that motor neurons have a high metabolic demand and that different subtypes of motor neurons (FF or S) have different vulnerability to energetic stress.

Conclusions: Using a realistic computer model, we studied the relationship between functional activity (firing properties) and metabolic energy requirements. The model is unique in that it includes ionic channels together with intracellular metabolic pathways linked to mitochondrial function, ATP synthesis and consumption, and pumps and buffers. We describe an instability triggered by mitochondrial failure that leads to a persistent energy deficit through a positive feedback loop, and that may be involved in the selective degeneration of fast motor neurons in ALS.

P151 A SMALL MOLECULAR COMPOUND CPN-9 SELECTIVELY PROTECTS AGAINST OXIDATIVE STRESS-INDUCED CELL DEATH BY ACTIVATING THE NRF2-ARE PATHWAY

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Keywords: neuroprotectant, Nrf2, oxidative stress

Background: Several lines of evidence have indicated that oxidative stress is implicated in the pathogenesis of many neurodegenerative diseases including amyotrophic lateral sclerosis (ALS). Thus, anti-oxidative stress therapy is thought to be one of the promising remedies for those diseases. Recently, we have shown that a small-molecule neuroprotectant CPN-9 selectively protects oxidative stress-induced cell death *in vitro,* and suppresses disease progression in an ALS mouse model (*SOD1*-H46R). However, the molecular mechanism by which CPN-9 selectively protects cells from oxidative stress remains unclear.

Objectives: To define the molecular signaling pathway for the CPN-9-mediated anti-oxidative stress activity.

Methods: We used human neuroblastoma SH-SY5Y cells differentiated by retinoic acid. To evaluate the effects of CPN-9 on the expression of the antioxidant stress genes and their products as well as on the cell viabilities, we performed RT-PCR, Western blotting, RNAi-mediated gene silencing, and *in vitro* cell viability assay.

Results: We first examined whether CPN-9 activates the transcription factor Nrf2 that promotes several antioxidant and detoxifying enzyme gene expression. CPN-9-treated SH-SY5Y

cells revealed an increase in the nuclear Nrf2 levels, indicating the activation of Nrf2 in cells. Several antioxidant and detoxifying enzymes including heme oxygenase-1 (HO-1), NAD(P)H quinone oxidoreductase 1 (NQO1), and glutamate-cysteine ligase modifier subunit (GCLM), whose expressions were regulated by Nrf2, were indeed significantly upregulated in SH-SY5Y cells by CPN-9. Consistently, the cytoprotective effect of CPN-9 was suppressed either by HO-1, NQO1, or GCL inhibitor. N-acetylcysteine prevented the CPN-9-induced nuclear translocation of Nrf2 with a concomitant attenuation of the CPN-9-mediated protection against oxidative stress. Further, knockdown of Nrf2 expression also resulted in decreased levels of HO-1, NQO1 and GCLM induced by CPN-9 and abolished its anti-oxidative stress activity, indicating that the Nrf2-antioxidant response element (ARE) pathway is essential for the CPN-9 mediated cytoprotectivity.

Discussion and conclusion: The present study showed that CPN-9 suppressed oxidative stress selective cell death via the activation of the Nrf2-ARE pathway with a concomitant induction of the antioxidant and detoxifying enzymes, suggesting that the Nrf2-ARE pathway serves as a potential neuroprotective drug target. Thus, CPN-9, the Nrf2-ARE activating compound, might represent a promising new therapeutic agent for ALS and other neurodegenerative diseases.

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P152 DEFECTIVE REGENERATION OF OXIDATIVELY-INACTIVATED 2-CYS PEROXIREDOXINS IN SOD1-RELATED AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: peroxiredoxins, oxidative stress, SOD1

Background: Peroxiredoxins (Prxs) are abundant redox-sensitive anti-oxidant enzymes that reduce hydrogen peroxide (H_2O_2) to water, becoming oxidized themselves. Oxidized 2-Cys Prxs may either be returned to their reduced state by thioredoxin or become overoxidized. Overoxidized 2-Cys Prxs ($PrxSO_{2/3}$) lose their hydroperoxidase activity but may then form multimers with a molecular chaperone function. Overoxidation of 2-Cys Prxs may be reversed by Sulfiredoxin-1. Previous studies demonstrated altered Prx levels in SOD1-ALS. This and the relevance of Prx anti-oxidant and protein chaperone roles to extant pathogenetic hypotheses led us to ask whether the oxidation state of the 2-Cys Prxs might be altered in ALS.

Objectives: We hypothesized that 2-Cys Prxs might spend longer in a more oxidized state in ALS and that this could have implications for disease pathogenesis and/or progression. We aimed to test this hypothesis in models of SOD1 ALS.

Methods: Fibroblasts were obtained from patients with I113T SOD1-related familial ALS and age and sex-matched controls. Western blotting for total 2-Cys Prxs, $PrxSO_{2/3}$, Sufiredoxin-1 and Sestrin 2 (previously thought to reduce $PrxSO_{2/3}$) was performed on cells grown under basal conditions and after exposure to 15 min 300mM H_2O_2. Stress recovery experiments (15 min 300mM H_2O_2, washout, 26 hours recovery) were performed with and without cycloheximide. Whole brain and spinal cord homogenates from G93A-overexpressing transgenic mice were also examined for $PrxSO_{2/3}$.

Results: No difference in total 2-Cys Prxs was found between patient and control fibroblasts and minimal $PrxSO_{2/3}$ was detected in either under basal conditions. Exposure to 300mM H_2O_2 for 15 min overoxidized 2-Cys Prxs to saturation with no detectable difference between patient and control cells. $PrxSO_{2/3}$ recovery was, however, significantly delayed in patient fibroblasts. Sulfiredoxin-1 induction after H_2O_2-treatment was both delayed and reduced in patient fibroblasts compared to controls. Both Sulfiredoxin-1 induction and $PrxSO_{2/3}$ recovery were abolished by cycloheximide pre-treatment. $PrxSO_{2/3}$ was almost undetectable by Western blotting in whole cord or brain preparations from G93A or non-transgenic littermate mice.

Discussion and conclusions: Delayed $PrxSO_{2/3}$ recovery after oxidative stress in I113T-FALS fibroblasts implies that 2-Cys Prxs spend longer in an oxidatively-inactivated state after oxidative challenge. Defence against H_2O_2 will be impaired for longer after each challenge suggesting the possibility of an above-normal rate of accumulation of oxidative damage with repeated exposures. Our results thus far suggest that the delay in $PrxSO_{2/3}$ regeneration may be due to both reduced and delayed Sulfiredoxin-1 induction after oxidative stress, an induction dependent upon protein translation. The underlying cause and functional effect of this deficit are currently under investigation. We further plan to address whether this effect can be seen in other forms of familial and sporadic ALS and, more importantly, whether it is also a feature of motor neuronal cells.

P153 EXCITOTOXICITY IN CULTURED MOTOR NEURONS

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Keywords: excitotoxicity, axon degeneneration, neuromuscular junction

Background: ALS is characterised by degeneration of both motor neurons and the neuromuscular junction. Although the causes of this degeneration remain largely unknown, evidence has strongly implicated glutamate excitotoxicity as a primary cause of motor neuron degeneration. However, it is currently unclear whether this toxicity is directed to the neuronal soma or via the neuromuscular junction.

Objectives: We aim to investigate the hypothesis that "somatodendritic excitotoxin exposure can result in primary degeneration of the distal axon". We are investigating this hypothesis by utilising a variety of motor neuron cell culture models, including established and novel techniques.

Methods: Primary cultures of spinal motor neurons were derived from E15 embryonic Sprague Dawley rats. Additionally, primary spinal glia and skeletal muscle cultures were obtained from neonates. Motor neurons were cultured onto either glial or muscle monolayers, or into compartmented chambers (Xona Microfluidics) containing proximal spinal glia and distal skeletal muscle. Cultures were fixed and characterised using standard immunocy to chemical techniques.

Results: Firstly, established culture techniques were evaluated, indicating spinal motor neurons grown on muscle feeder layers exhibit different growth characteristics from motor neurons cultured on glial cells. At 7 days *in vitro* (DIV) motor neurons demonstrated a significant ($p < 0.05$) increase in mean axon length ($n = 5$, 1363 μm ± 104 μm) when compared to glial cultured neurons ($n = 5$, 759 μm ± 1054 μm), and significantly ($p < 0.05$) reduced mean number of neurites when cultured on muscle monolayers ($n = 5$, 2.6 ± 0.3) compared with glial cultures ($n = 4$, 4.5 ± 0.8). By 21 DIV motor neurons cultured on both skeletal muscle and glial feeder layers were > 85% immunopositive for dephosphorylated neurofilaments, indicating relative culture maturity. Putative neuromuscular junctions were present in motor neuron-skeletal muscle co-cultures, indicated by clustering of acetylcholine receptors. Mean neuron survival was diminished when cultured directly onto skeletal muscle monolayers, evident from 3 DIV when compared with motor neurons on glial cells. Excitotoxicity was induced in both culture systems (25 μM kainic acid for 24 hours), resulting in significant ($p < 0.05$) and severe loss of motor neurons co-cultured with astrocytes ($n = 3$, 85% ± 1.8%) compared with motor neurons on a skeletal muscle monolayer ($n = 3$, 53% ± 6.3%). Currently, we are developing techniques to culture compartmented motor neurons with proximal glial cells and distal skeletal muscle.

Discussion and conclusions: These data indicate that a compartmented culture strategy may facilitate normal motor neuron development, and simultaneously allow focal excitotoxin exposure. This will enable investigation into the primary site of excitotoxic degeneration, providing insights into the mechanisms of degeneration in ALS.

P154 THE ROLE OF THE PHOSPHATIDYLINOSITIDE-3-KINASE PATHWAY AND INTRACELLULAR PH IN TDP-43 MODELS OF ALS

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Keywords: HIF-1α, SGK1, NHE1, PI3-kinase, TDP-43

Background: Hypoxia and chronic respiratory deficiency are associated with the clinical pathology of ALS, and have been shown to occur early in the disease process. Hypoxia-inducible factor 1 alpha (HIF-1α) is involved in activating various factors which play an integral role in hypoxia, with previous research indicating that the selective inhibition of HIF-1α may occur in ALS. HIF-1α is activated via the phosphatidylinositide-3-kinase (PI3-kinase) pathway, which also activates serum and glucocorticoid-inducible-kinase-1 (SGK1) in response to cell stress. SGK1 has been shown to be involved in the activation of various enzymes and transcription factors, and has also been found to be upregulated in a transgenic model of ALS. Research also indicates that SGK1 and the Na + /H + exchanger 1 (NHE1) genes are activated in response to hypertensive stress. NHE1's role is to maintain neutral intracellular (*i*) pH, as inhibition of NHE1 is associated with the acidification of pH*i* and neurodegenerative disease. ALS is also characterised by the presence of intracellular inclusions comprised of aggregated proteins, such as TAR DNA binding protein 43 (TDP-43). However, the relationship between HIF-1α, SGK1, NHE1 and TDP-43 has not been explored.

Objectives: To investigate the association between HIF-1α, SGK1, NHE1 and TDP-43 using Pathway Studio.

Methods: Pathway Studio 8.0 (Ariadne Inc. Rockville MD) will be used to analyse the pathways between HIF-1α, SGK1, NHE1 and TDP-43.

Results: Mutant TDP-43 was found to be associated with HIF-1α through a mutual binding to glutathione-s-transferase. SGK1 expression was found to be associated with COMMD1 which in turn binds with the glutathione-s-transferase/ ubiquitin pathway. NHE1 function was associated with TDP-43 through Zinc (Zn^{2+}) and the caspase pathway.

Discussion and conclusions: Results indicate that HIF-1α binds to the factor inhibiting HIF-1α (FIH-1) region on glutathione-s-transferase leading to a downregulation of HIF-1α, while TDP-43G37V was also shown to bind to glutathione-s-transferase. SGK1 was associated with COMMD1, with a knockdown of COMMD1 leading to an increased expression of SGK1. COMMD1 may be of significance in relation to TDP-43 as it binds to the glutathione-s-transferase/ ubiquitin proteasomal system and is stated to be involved in ensuring protein stability. NHE1 was shown to be regulated by Zn^{2+} with an increase in extracellular Zn^{2+} associated with the aggregation of endogenous TDP-43. NHE1 and TDP-43 were also associated with the caspase pathway, with an increase in caspase activation linked to TDP-43, and with caspase inhibition being related to the rescue of NHE1 expression. NHE1 may be involved in cell survival via a decrease in caspase activity and the activation of the PI3-kinase pathway. Therefore, factors in the PI3-kinase pathway, such as HIF-1α and SGK1, as well as the regulation of pH*i* may play a significant role in the development of TDP-43 related pathologies.

P155 ANALYSIS OF THE VASCULAR ENDOTHELIAL GROWTH FACTOR SYNTHETIC PATHWAY AND NUCLEOCYTOPLASMIC TRANSPORT IN SPINAL ANTERIOR HORN CELLS IN ALS AND MSOD1G93A TRANSGENIC MICE MODELS

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Keywords: VEGF, HIF1-α, karyopherin β1, nucleocytoplasmic transport

Background: The vascular endothelial growth factor (VEGF) exerts neuroprotective effects on motor neurons. However, the role of VEGF expression and nucleocytoplasmic transport in the pathogenesis of amyotrophic lateral sclerosis (ALS) remains to be clarified.

Object: To investigate the role of the VEGF synthetic pathway and nucleocytoplasmic transport in spinal anterior horn cells in the pathogenesis of ALS by using ALS and G93A mutant superoxide dismutase 1 (mSOD1) transgenic (Tg) ALS model mice.

Method: We performed immunohistochemical analysis of molecules involved in VEGF pathway and nucleocytoplasmic transport in mSOD1^{G93A} Tg mice, together with six postmortem ALS cases and six non-neurological disease controls.

Result: The immunoreactivity of VEGF receptors—VEGFR1 and 2—in the spinal anterior horn cells was significantly weaker in ALS cases than in the controls. In contrast, immunoreactivity of hypoxia-inducible factor (HIF)-1a in the anterior horn cells was significantly stronger in ALS cases than in the 9 controls. Nuclear staining of HIF-1a was observed only in several shrinking neurons. VEGF immunoreactivity in the anterior horn cells was also weaker in mSOD1^{G93A} Tg mice than in non-transgenic mice aged 12 (presymptomatic stage) to 18 weeks (terminal stage), despite of the stronger HIF-1a immunoreactivity in mSOD1^{G93A} Tg mice than in non-Tg mice. Nuclear staining of HIF-1a was detected in anterior horn cells after 16 weeks (early symptomatic stage); nonetheless, VEGF immunoreactivity in motor neurons continued to be weaken with age, whereas that in reactive astrocytes was more enhanced in mSOD1^{G93A} Tg mice than in non-transgenic mice. HIF-1a transports to nucleus by NLS system, such as Karyopherin b1. Double-fluorescence staining showed that HIF-1a co-localized with Karyopherin b1 in the cytoplasm of anterior horn cells in mSOD1^{G93A} Tg mice. Furthermore, nuclear staining in anterior horn cells was also weaker in mSOD1^{G93A} Tg mice than in non-transgenic mice aged 12 weeks (presymptomatic stage). Also as the disease progressed, discontinuous staining of nuclear membrne with Karyoperinb1 was increased. These intend to dysfunction nucleocytoplasmic transport.

Conclusion: These results indicate that pathogenesis of sporadic ALS ,as well as mSOD1^{G93A} Tg model mice, involves down-modulation of VEGF receptors and dysfunction of nucleocytoplasmic transport from the presymptomatic stage.

P156 GABA-INDUCED CURRENTS ARE UP-MODULATED BY MONOCYTE CHEMOATTRACTANT PROTEIN-1 IN CORTICAL NEURONS FROM THE G93A MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: GABAa receptor, MCP-1, cortex

Background: The inflammatory response in Amyotrophic Lateral Sclerosis (ALS) is well documented. Pathological analysis shows that the neurodegenerative process is accompanied by microglial activation and astrogliosis. Microglia, as the main immunocompetent cell type of the Central Nervous System (CNS), have the potential to secrete most of the inflammatory mediators that could contribute to disease progression. Some of these have been shown to be elevated in the cerebrospinal fluid (CSF) and CNS tissues of ALS patients, like the monocyte chemoattractant protein-1 (MCP-1). Interestingly, it has been described that the chemokine MCP-1 is able to alter the electrical activity of nociceptive neurons, indicating a modulatory effect on ionic channels involved in neuronal excitability.

Objectives: Since it has been reported that Cl^- influx through the ionotropic $GABA_A$ receptors could be harmful to neurons and that in G93A spinal motor neurons an altered $GABA_A$ receptor subunit composition has been observed, the aim of this work was to investigate whether MCP-1 was able to modulate GABA-induced currents both in control and G93A cultured cortical neurons.

Methods: Electrophysiological recordings were performed on 8-10 day-old *in vitro* cortical neurons from control and G93A mice, using the patch-clamp technique in whole cell configuration. Tetrodotoxin (1μM), cadmium (100 μM) and D-2-amino-5-phosphonovaleric acid (10 μM) were added to the external solution to block voltage-gated Na^+ and Ca^{2+} channels and N-methyl-D-aspartic acid receptors, respectively. GABA and MCP-1 were dissolved in extracellular solution and applied with a fast multibarrel pipette perfusion system controlled by electronic valves. The currents induced by GABA perfusion (5 μM) were recorded in control condition and during MCP-1 (100 ng/ml) perfusion, both in neurons from Control and G93A mice.

Results: GABA-induced currents were induced by bath application of GABA and the current amplitudes resulted significantly higher following MCP-1 perfusion (100 ng/ml), both in Control ($n = 22$; $p < 0.01$) and G93A ($n = 27$; $p < 0.001$) neurons. Moreover, the increase of the GABA-induced current was significantly higher in G93A neurons than Control ($p < 0.05$). The MCP-1 effect was not mediated by its G-protein coupled receptor, CCR2, because GABA currents were still higher both in Control ($n = 8$) and G93A neurons ($n = 9$) when the BMS-CCR2-22 (1 μM), a CCR2 antagonist, or when GDP-βS (2 mM), the G-proteins' inhibitor, were added to the external and internal solutions, respectively.

Discussion and conclusions: These results suggest that: *i)* MCP-1 is able to modulate the GABA-induced currents both in control and G93A cortical neurons by increasing the amount of cloride influx; *ii)* in G93A neurons the increase of the cloride influx is significantly higher compared to controls and *iii)* GABA-induced current modulation is not mediated by CCR2 receptor activation. Further studies will be necessary to ascertain the MCP-1 mechanism of action on $GABA_A$ receptors and the role of MCP-1 in ALS pathology.

P157 LOW-DOSE THAPSIGARGIN AND WILD-TYPE SOD-1 SYNERGISTICALLY ENHANCES NEURITE GROWTH IN MOUSE MOTONEURONAL CELLS

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Keywords: thapsigargin, NSC-34 cell, SOD1

Background: Thapsigargin (TG), an inhibitor of the sarcoendoplasmic reticulum Ca^{2+}-ATPase, modulates intracellular concentration of Ca^{2+} in a time- and compartment-dependent manner. TG at micromolar concentrations has been used as an apoptosis-inducing agent in cellular models of amyotrophic lateral sclerosis (ALS). However, cellular effects of TG at nanomolar concentrations have not been evaluated in models. We observed that nanomolar levels of TG enhanced neurite growth in mouse motoneuronal NSC-34 cells without inducing apoptosis. This finding led us to test whether TG-enhanced neurite growth is affected by overexpressed SOD-1, considering that SOD-1 is involved in intracellular regulation of Ca^{2+} concentration. After confirming overexpressed SOD-1 affects TG-enhanced neurite growth, we are

currently investigating whether spatiotemporal change of intracellular Ca^{2+} concentration is a molecular mechanism underlying synergistical enhancement of neurite growth by low-dose thapsigargain and overexpressed SOD-1.

Objectives: To determine whether overexpressed SOD-1 affects low-dose TG-enhanced neurite growth in NSC-34 cells, and to understand the underlying molecular mechanism

Methods: NSC-34 cells were transfected with GFP, or GFP-tagged wild-type SOD1 (WT) or GFP-tagged mutant SOD1 with G93A constructs, and then differentiated with serum withdrawal. After 1 day, TG at different concentrations (3, 30, 300 nanomole) was treated to cells for 20 minutes. After 1 day, cells were fixed and stained with neurite-specific antibodies. Neurite growth of each cell was determined by measuring total lengths of neurites, the sum of the lengths of all neurites from each cell, using Image J software with Neurite Tracer. For in vivo Ca^{2+} imaging, Rhod-3 and Rhod-2 were used for the visualization of intracellular Ca^{2+}. Data were analyzed by Mann-Whitney test or by one-way ANOVA. Differences were considered significant at the $p < 0.05$ level.

Results: Quantitative analysis of neurites growth demonstrated that 30 nanomolar TG treatment increased total neurite length, whereas the other TG treatments failed to increase the length (GFP 0nM: 154.15 ± 6.2; 3nM: 170.52 ± 16.31; 30nM: 218.19 ± 23.23; 300nM: 126.51 ± 8.04, G93A 0nM: 143.55 ± 15.7; 3nM: 168.35 ± 25.80; 30nM: 190.23 ± 12.4; 300nM: 119.02 ± 4.83, WT 0nM: 151.46 ± 11.84; 3nM: 173.31 ± 16.84; 30nM: 235.4 ± 25.68; 300nM: 141.27 ± 10.09 (unit = micrometer, $n = 10$, $p < 0.05$)). The analysis also demonstrated that in the cells treated with 30 nanomolar TG, the cell overexpressing WT contain increased level of neurite growth compared with the cells overexpressing G93A or GFP (GFP 30nM: 218.19 ± 23.23, G93A 30nM: 190.23 ± 12.4, WT 30nM: 235.4 ± 25.68 (unit = micrometer, $n = 10$, $p < 0.05$)).

Discussion and conclusion: We found that low-dose, 30 nanomolar, TG increased neurite growth in NSC-34 cells, and that this effect was accelerated by WT, but not G93A. These findings suggest that WT and G93A are differentially involved in the intracellular Ca^{2+} regulation, and thus the identification of how WT and G93A affect the low-dose TG-mediated spatiotemporal change of intracellular Ca^{2+} concentrations would shed light on the pathogenic mechanism of ALS.

P158 NEUROTOXIC PATHWAYS *IN VITRO* – NEURORESCUE EFFECT OF RILUZOLE ON THAPSIGARGIN(THAPS), BUT NOT STAUROSPORINE(STS), HYDROGEN PEROXIDE(H_2O_2) AND HOMOCYSTEINE(HCY) TOXICITY IN DIFFERENTIATED MOUSE MOTOR NEURON-NEUROBLASTOMA HYBRID(NSC-34D) CELLS

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Keywords: riluzole, drug development, pathogenetic pathways

Background: An *in vitro* model was developed to examine the pathways underlying ALS pathogenesis using a panel of neurotoxins and the differentiated form of the NSC-34 cell line, NSC-34D. Each of these compounds has a mechanism of action which is potentially involved in the molecular pathogenetic pathways found in ALS. We have previously shown neuroprotection in this model system (1,2). Other groups have shown neuroprotection in vitro by riluzole against excitotoxins (3) and STS (4). As ALS patients begin their treatment when some pathways are already activated, we have moved to a new paradigm, neurorescue, where neurotoxicity occurs simultaneous with the therapeutic agent being examined. Our goal is to examine the new neurorescue paradigm with each neurotoxin with respect to mechanism to analyze treatment effect size as well as potentiation of this treatment effect size *in vitro.*

Objective: We utilized the NSC-34D cells to examine the effects of riluzole on cell death induction by STS, Thaps, H_2O_2 and HCy (5).

Methods: Nuclear morphology, caspase-3/7 activation and high content imaging were used to assess toxicity of these compounds with and without co-treatment with riluzole.

Results: STS was the most potent compound at killing NSC-34D cells with a toxic concentration at which 50% of maximal cell death is achieved($TC_{50} = 10$ nM), followed by Thaps($TC_{50} = 0.9$ μM) and H_2O_2($TC_{50} = 15$ μM). HCy required higher concentrations to kill at the same level($TC_{50} = 2.2$ mM)(5). Using the neurorescue paradigm in NSC-34D cells, we show that riluzole provides neurorescue against Thaps-induced cell death (by 20.3%; $42.7 + 2.7$ vs. $63.0 + 3.0$; $p < 0.05$) but had little to no effect on STS-, H_2O_2 - and HCy-induced cell death. This effect of riluzole on cell death induction was independent of caspase-3/7 activation. Further analysis in a pilot study utilizing high content analysis revealed that toxicity with STS could be observed continuously over 24 h in a dose-dependent manner with no early or late benefit of riluzole on STS toxicity.

Conclusions: Riluzole, in a neurorescue paradigm associated with co-administration of riluzole at the initiation of neurotoxin exposure, is capable of reducing cell death, independent of caspase-3/7 activation, only of Thaps neurotoxicity but not STS, H_2O_2, or HCy neurotoxicity.

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THEME 9 HUMAN CELL BIOLOGY AND PATHOLOGY

P159 QUANTIFICATION OF GSK-3ß IN PERIPHERAL BLOOD MONONUCLEAR CELLS IN ALS PATIENTS

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Keywords: GSK-3ß, peripheral blood mononuclear cells, biomarker

Background: The glycogen synthase kinase-3β (GSK-3β) phosphorylates many metabolic, signalling, and structural proteins, thereby regulating the neuronal plasticity, gene expression, and cell survival. GSK-3β is known to be altered in nerve tissue in Alzheimer's and ALS, but little is known about its possible alteration in peripheral blood mononuclear cells (PBMCs) and studies published in Alzheimer's are inconclusive.

Objective: The aim of our work was to quantify the total GSK-3β (tGSK), PSer9-GSK-3β (pGSK) and ratio pGSK/tGSK in PBMSs from ALS patients.

Methods: Sixteen healthy controls (HC) and 22 ALS patients (PALS) were studied. Among patients, 14 were clinically probable ALS (PpALS) and 8 clinically definite ALS (PdALS). PBMCs were separated from whole blood using BD Vacutainer CPT tubes (Becton Dickinson). Once washed, cells were lysed in 600 µL of RIPA buffer and a cocktail of protease inhibitors. Fifty microliters of the lysate were tested for tGSK and pGSK using commercial tests (Enzo Life Sciences). Total protein was measured using a commercial BCA test (Pierce). Quantitative data are shown as median and interquartile range (IQR), in µg/mg, and compared using the non parametric Mann-Whitney test.

Results: No significant differences were found in tGSK between HC (46.85, IQR = 43.72-50.3) and PALS (44.45, IQR = 39.67-55.72), nor among HC and PpALS (45.6, IQR = 36.7-55.72) and PdALS (44.45, IQR = 40.72-70). We neither found significant differences in pGSK between HC (9.5, IQR = 7.75-12.12) and PALS (10.15, IQR = 8.32-13.8), nor among HC and PpALS (10.15, IQR = 8.62-13.8) and PdALS (10.0, IQR = 5.25-19.47). In pGSK/tGSK we neither found significant differences when comparing HC (0.21, IQR = 0.16-0.26) and PALS (0.24, IQR = 0.19-0.29) or PdALS (0.21, IQR = 0.15-0.26) but, although neither were significant (p = 0.08), we found differences when comparing HC with PpALS (0.25, IQR = 0.19–0.33).

Discussion: We found no significant differences in PBMCs tGSK, pGSK or pGSK/tGSK among healthy controls, PALS, neither in PpALS nor in PdALS, although we found a tendency for increased values of pGSK/tGSK in PpALS. As GSK-3β is inactivated by phosphorylation at Ser9, our results may indicate that the GSK-3β activity is decreased in PpALS, although its total levels are not modified. Our results suggest that PBMC GSK-3β levels do not correlate with the high expression observed in nerve tissue of PALS. To date, no studies on GSK-3β levels in PBMCs of PALS have been reported.

Conclusions: No significant differences in tGSK, pGSK or pGSK/tGSK of PBMCs are found among healthy controls, probable ALS and ALS patients. A non-significant tendency to increased values of pGSK/tGSK is found in early stages of ALS.

P160 GLYCOGEN SYNTHASE KINASE-3ß LOCALIZES TO THE CYTOPLASMIC INCLUSIONS IN THE SPINAL MOTOR NEURONS IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: GSK3ß, TDP-43, inclusion body

We immunohistochemically examined glycogen synthase kinase-3β (GSK-3β) expression in the spinal cord in amyotrophic lateral sclerosis (ALS). Spinal cords were obtained from 13 patients with ALS, and 8 controls without neurological disease. Specimens were fixed in 15% neutral formalin for 2 weeks, and then embedded in paraffin. Axial sections (5-µm) of the lumbar spinal cords were excised for immunohistochemistry. Immunostaining was done with rabbit polyclonal antibodies against GSK-3β, Ser9-phosphorylated GSK-3β at (pGSK-3β), TDP-43, Ser409/410-phosphorylated TDP-43 (pTDP43). GSK-3β was localized in the punctate structures in the cytosol of motor neurons in the control spinal cord. GSK-3β-immunoreactive (IR) puncta were reduced in ALS. GSK-3β was present in cytoplasmic inclusions (CIs) such as round inclusions and was less frequent in skein-like inclusions. GSK-3β colocalized with TDP-43 in the CIs. GSK-3β-IR CIs were mainly found in neurons without GSK-3β-IR puncta. Our results suggest that aggregation of GSK-3β-IR CIs correlated with the decrease in GSK-3β-IR puncta. Accumulation of GSK-3β in the CIs may be toxic to ALS motor neurons.

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P161 THE KYNURENINE PATHWAY AND INFLAMMATION IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: kynurenine pathway, excitotoxicity, neuroinflammation

ALS is a motor neuron degenerative disease and the kynurenine pathway (KP) is emerging as a possible cause. The KP metabolizes tryptophan (TRP) and generates neuroactive compounds, picolinate (PIC) and quinolinate (QUIN). The first enzyme is indoleamine-2,3 dioxygenase (IDO-1). This study aims were to characterize the KP in ALS patients (*ex vivo*) and NSC34 motor neuron cell line (*in vitro*).

GC/MS and HPLC were used to analyze CSF and serum for QUIN, PIC, TRP and kynurenine levels of ALS patients (n = 150) and controls (n = 20). Antibodies to HLA-DR, IDO and QUIN were used on paraffin embedded ALS spinal cord and motor cortex. In NSC34 cells, RT-PCR and ICC were used to characterize KP enzymes and catabolites; LDH test assessed the effect of QUIN, with and without inhibitors.

Results showed significant increases in CSF and serum TRP ($P < 0.0001$), KYN ($P < 0.0001$) and QUIN ($P < 0.05$) and decrease serum PIC ($P < 0.05$) in ALS samples. Significant numbers of activated microglia-expressing HLA-DR ($P < 0.0001$) and increase in neuronal and microglial expression of IDO and QUIN were detected in ALS motor cortex and spinal cord. NSC34 cells stained positive for KP enzymes and catabolites; RT-PCR showed the presence of most of KP enzymes; and LDH production showed a dose dependant increase with QUIN, partially inhibited by antagonists.

Our results indicate the presence of neuroinflammation in ALS and provide the first strong evidence for the involvement of the KP in the neuropathogenesis of ALS.

P162 ELEVATION OF ALTERNATIVE MACROPHAGE ACTIVATION MARKER PARC/CCL18 IN PLASMA FROM PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: inflammation, alternative macrophage activation, PARC/CCL18

Background: Inflammation and immune activation are associated with ALS; however, the relevance of these processes to pathogenesis has not been adequately explored. Monocyte/macrophages (MO) are critical mediators of inflammatory processes, and play a major role in the resolution of inflammation by producing anti-inflammatory cytokines and chemokines as well as eliminating tissue debris. MO activation comprises two basic patterns: classically activated or type I MO, which are pro-inflammatory effectors, and alternatively activated or type II MO that exhibit anti-inflammatory properties. Involvement of classical MO activation in ALS pathogenesis has been confirmed by various investigations. Our recent studies on gene expression in peripheral blood mononuclear cells from patients with ALS showed upregulation of both classic type I interferon-induced genes and type II alternative MO activation genes, suggesting a hybrid activation state that implicates both classical and alternative MO activation in ALS pathogenesis(1). In order to examine whether alternative MO activation observed by gene expression profiling would be confirmed in ALS patients, we evaluated the alternative MO activation marker, PARC/CCL18 in plasma from patients with ALS.

Objectives: 1) To quantify levels of plasma PARC/CCL18 in ALS patients as compared to healthy controls; 2)To determine if plasma levels of PARC/CCL18 correlate with clinical stage of disease in ALS.

Methods: PARC/CCL18 ELISA was performed to quantify plasma levels of PARC/CCL18 in heparinized blood samples from 20 ALS patients (Forbes-Norris MDA/ALS program, CPMC) and 20 healthy controls (HC). Results from this immune study were evaluated in light of the duration of disease or severity of neurological impairment as determined by ALSFRS-R score.

Results: Compared to HC (43.2 ± 26.5ng/ml), significantly elevated levels of plasma PARC/CCL18 were identified in patients with ALS (101.5 ± 83.4ng/ml, $p = 0.0069$). Plasma PARC/CCL18 levels were independent of ALS disease severity as defined by ALSFRS-R score. However, there was a positive correlation between plasma PARC/CCL18 levels and disease duration in ALS ($r = 0.7756$, $p < 0.0001$). Significantly higher levels of plasma PARC/CCL18 were found in ALS patients with the lowest rate of disease progression (DP rate: loss of ALSFRS-R score/month) (DP rate < 1: 119.9 ± 93.4ng/ml, n = 14) as compared to those with the active disease progression (DP rate > 1: 58.5 ± 24.3ng/ml, n = 6) ($p = 0.0234$).

Conclusions: This study, for the first time, revealed that plasma levels of alternative MO activation marker PARC/CCL18 were significantly higher in ALS patients, and that increased PARC/CCL18 levels correlated with the duration/rate of disease progression of ALS. These data suggest that alternative MO activation might be important for survival during ALS disease process by countering pathogenic signaling by classically activated type I MOs. These findings provide new insights into the pathogenesis of chronic MO activation in ALS, and identify a new potential candidate marker for tracking ALS disease progression.

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P163 MUTANT FUS INDUCES ENDOPLASMIC RETICULUM STRESS IN AMYOTROPHIC LATERAL SCLEROSIS AND INTERACTS WITH PROTEIN DISULPHIDE ISOMERASE

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Keywords: FUS fused in sarcoma/ translocated in liposarcoma, endoplasmic reticulum stress, protein disulphide isomerase

Background: Endoplasmic reticulum (ER) stress is increasingly recognized as an important and early pathway to motor neuron death in animal and cellular disease models based on superoxide dismutase 1 (SOD1). ER stress involves upregulation of an important ER chaperone, protein disulphide isomerase (PDI), which is protective against mutant SOD1 aggregation and toxicity.

Objective: Mutations in the gene encoding fused in sarcoma/ tranlocated in liposarcoma (FUS) are linked to amyotrophic lateral sclerosis (ALS), but the mechanisms by which these mutants trigger neurodegeneration remain unknown. FUS is normally located in the nucleus but in sporadic and familial ALS, FUS redistributes to the cytoplasm and forms inclusions, similar to TAR DNA binding protein 43. In this study, we investigated the effect of nuclear and cytoplasmic mutant FUS on ER stress.

Methods: A motor neuron like cell line (NSC-34) was transiently transfected with wildtype and two mutant FUS proteins. Activation of ER stress and ER localisation was assayed by immunocytochemistry and immunoprecipitation. *Postmortem* spinal cords from two ALS patients and a control patient without any neurological disorders were immunostained with both anti-FUS and anti-PDI antibodies.

Results: We demonstrated that mutant FUS, which mislocalises to the cytoplasm, but not nuclear mutant FUS, specifically triggers ER stress in NSC-34 cells. This was determined by activation of IRE1 and ER stress-specific pro-apoptotic C/EBP homologous protein (CHOP). Mislocalised mutant FUS co-localised with markers of the ER, indicating that redistribution to the cytoplasm is associated with the ER. Furthermore, mutant FUS also co-localised with PDI in NSC34 cells and PDI was co-localised with FUS inclusions in human ALS lumbar spinal cords, in both sporadic ALS or mutant FUS-linked familial ALS tissues.

Conclusions: These findings implicate ER stress in the pathophysiology of FUS and suggest a possible role for PDI in the misfolding of FUS. This study also provides evidence for common pathogenic pathways in ALS linked to the ER.

P164 AUTOPHAGY-RELATED PROTEINS IMMUNOREACTIVITY IN BASOPHILIC INCLUSION BODY DISEASE

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Keywords: autophagy, basophilic inclusion, stress granules

Background: Basophilic inclusions (BIs) were originally observed in patients with juvenile amyotrophic lateral sclerosis (ALS) and were then found also in adult-onset motor neuron disease with BIs. In a previous immunohistochemical study, we obtained the first evidence for the presence of RNA and certain mRNA-related proteins in BIs, suggesting a link between BIs and stress granules (SGs). However, the exact constituents of BIs and process of formation of these inclusions are yet unknown. Recently, there has been a growing interest in the role of the autophagy-lysosome pathway in neurodegenerative diseases. However, the role of autophagy in BI formation has not yet been studied.

Objective: We studied the involvement of several autophagy-related proteins in BI formation, as well as in several inclusions in other motor neuron disease.

Methods: Applying antibodies specific for autophagy-related proteins, we investigated the neuronal inclusions in the motor cortex and spinal cord in 2 patients with basophilic inclusion body disease (BIBD), in 5 with sporadic amyotrophic lateral sclerosis (SALS), and in 1 with familial ALS with a Cu/Zn superoxide dismutase (SOD1) mutation (FALS) as well as in G93A mutant SOD1 transgenic mice.

Results: In BIBD specimens, BIs showed strong immunoreactivity for microtubule-associated protein 1 light chain 3 (LC3), autophagy-related gene (Atg) proteins, p62, and histone deacetylase 6 (HDAC6). However, BIs were not immunoreactive with antibodies specific for cathepsin D (catD) or lysosomal membrane protein 2 (LAMP2). The other inclusions in other motor neuron diseases, e.g., skein-like inclusions, Bunina bodies, round inclusions, spheroids, conglomerate inclusions or Lewy body-like hyaline inclusions, were not positive for LC3, Atg proteins or HDAC6.

Conclusions and discussion: LC3, p62, Atg, and HDAC6 proteins are effectors essential before the completion of autophagosome formation, whereas catD and LAMP2 are elements that appear after the fusion of autophagosomes with lysosomes. The results of our present study indicate that the autophagy was disrupted before the completion of autophagosome formation in these BIBD patients. Based on the results of our previous and present studies taken together, the following scenario might be plausible: SGs, which aggregate in response to an unidentified stress, are tagged with p62 and initially processed for autophagy. However, defective autophagy accelerates the aggregation of SGs by HDAC6, resulting in the formation of BIs in this disease.

P165 AN IMMUNOHISTOCHEMICAL STUDY OF UBIQUITIN IN THE SKIN OF SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: immunohistochemical study, ubiquitin, skin

Background: Ubiquitin (UB) is implicated in non-lysosomal degradation of short-lived and abnormal proteins and can be demonstrated as a part of many intraneuronal inclusions such as the Lewy body, the Pick body, the neurofibrillary tangle, and the argyrophilic inclusion in multiple system atrophy. The disease-causing proteins in many chronic degenerative conditions have a propensity to form intracellular aggregates containing the ubiquitinated proteins. UB-immunoreactive filamentous inclusions, previously undetected with routine histological methods, have been found in spinal anterior horn cells of patients with amyotrophic lateral sclerosis (ALS) and it has been suggested that they may be characteristic of these disorders. It is unknown, however, whether UB-positive structures are present in ALS skin.

Objectives: We have carried out an immunohistochemical study of UB in skin from ALS patients.

Methods: Skin biopsy samples were taken from the upper left arm of 19 patients with ALS (mean age 61.5 years) and from 21 controls with other neurodegenerative diseases matched for sex and age (mean age 62.1 years). Routine formalin-fixed paraffin-embedded 6 μm sections were immunostained according to standard techniques. The sections were incubated with anti-UB antibody. After washing in phosphate-buffered saline, biotinylated anti-IgG was applied. The sections were stained by ABC kit. The immunoreactivity was quantified with an image-analysis system. Statistical comparisons were made by the two-tailed Student's *t* test with $p < 0.05$ as the significance level. Correlation coefficients were calculated by the least-squares method. Results are expressed as the mean ± SD.

Results: The cytoplasm of UB-positive (UB^+) cells showed no positive immunoreaction. Numerous UB^+ cells were observed in the epidermis in ALS patients, which became more marked as ALS progressed, and a small number of cells were seen in controls. UB immunoreactivity of UB^+ cells was markedly positive in the epidermis and moderately positive in some dermal blood vessels and glands in ALS patients. These findings became more conspicuous as ALS progressed. On the other hand, UB + cells of the epidermis, dermal blood vessels and glands in control subjects showed a weak positive reaction even after repeated antigen-retrieval trials. The proportion of UB + cells in the epidermis in ALS patients (mean ± SD, 80.1 ± 15.1%) was significantly higher ($p < 0.001$) than in controls (4.4 ± 2.2%). There was a significant positive relationship ($r = 0.92$, $p < 0.001$) between the proportion and duration of illness in ALS patients, but there was no such relationship in control subjects.

Conclusions: The conspicuous finding in the skin of ALS was the increased UB immunoreactivity, which was even more significant with a longer duration of illness. These data suggest that changes of UB in ALS skin are related to the disease process and that metabolic alterations of UB may take place in the skin of patients with ALS.

P166 ALTERED TENASCIN-R EXPRESSION AND PERINEURONAL NET MORPHOLOGY IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: tenascin, perineuronal net, extracellular matrix

Background: Changes in the extracellular matrix are implicated in the pathogenesis of amyotrophic lateral sclerosis (ALS), yet the mechanisms leading to these changes are unclear. In the CNS, the extracellular matrix is highly specialized and that which surrounds neurons is referred to as a perineuronal net (PNN). The composition of PNN's is diverse, although previous work has established that the protein Tenascin-R (TNR) is necessary for PNN formation. We identified decreased levels of TNR in ALS patients using an unbiased proteomics approach and thus sought to further characterize levels of the protein in post-mortem tissue from ALS patients.

Objectives: This study had two principal goals. The first was to determine TNR levels in post-mortem tissue from ALS patients and normal controls. The second was to examine PNN morphology in control and ALS patients.

Methods: We obtained post-mortem spinal cord tissue (paraffin sections and snap frozen tissue) from the University of Pittsburgh ALS Tissue Bank. Normal controls were age-matched to their ALS counterparts. To determine TNR levels, we used a combination of immunoblot and immunohistochemistry using anti-TNR specific antibodies. PNN morphology was assessed by immunohistochemistry using biotinylated lectin from Wisteria Floribunda, a lectin conjugate commonly used to stain PNN's.

Results: Our immunohistochemical analysis of TNR in spinal cord tissue revealed a striking loss of normal TNR immunoreactivity around the cell body and processes of motor neurons in ALS patients. In contrast, the control group showed a robust and continuous staining pattern in these areas. This finding was verified by immunoblot analysis, which showed reduced levels of TNR in tissue extracts from ALS patients relative to controls. Our analysis of PNN morphology also revealed aberrant morphology in ALS, reflecting a loss of normal structure.

Discussion: Our immunohistochemistry and immunoblot results validate our CSF based proteomics analysis and demonstrate a distinct loss of TNR protein and immunoreactivity in the spinal cord of ALS patients. This loss of TNR expression is correlated with abnormal PNN morphology. Collectively, these results suggest that alteration to the extracellular matrix surrounding motor neurons is a component of ALS. Future studies will examine the role of TNR for maintenance of PNN's, as previous studies have shown that TNR is necessary for PNN formation. If TNR is essential for PNN maintenance around adult motor neurons, a loss of TNR expression could severely compromise the viability of motor neurons.

Conclusions: TNR protein level is decreased in post-mortem tissue from ALS patients compared to normal control tissues and abnormal PNN morphology is evident surrounding motor neurons. Further investigation is warranted to examine molecular mechanisms that regulate TNR expression and consequences for motor neuron survival.

P167 NEUROPATHOLOGICAL FINDINGS IN A PEDIGREE WITH ALTERNATING FRONTAL LOBE DEMENTIA AND AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: FALS, FTD, SOD1

Background: Ubiquitinated inclusions are hallmarks of most forms of frontotemporal dementia (FTD) and of amyotrophic lateral sclerosis (ALS). Pedigrees in which these syndromes alternate are also known. Both these facts point towards a shared pathophysiology between these syndromes. However, few autopsy reports are available from such pedigrees. Here we report findings from 6 patients from a pedigree with more than 2000 known members in 14 generations. Of these 39 patients have ALS or ALS-dementia while 12 have FTD.

Objectives: To characterize and systematically investigate clinical and morphological findings in six autopsies of ALS/ FTD patients.

Methods: Brain and spinal cord sections from six autopsies were investigated by immunohistochemistry. Four different anti-peptide antibodies with specificity for misfolded/disordered SOD1were used as well as antibodies directed at GFAP, ubiquitin, TDP-43, NF and cystatin C.

Results: The mean age of symptoms in the ALS/ ALS-dementia cases is 60.9 years and the mean duration of disease is 30 months. ALS patients in this family have the classical Charcot type of the disease. Of the six patients, 5 showed ALS/ALS-dementia and one had a pure FTD. In the ALS cases a loss of motoneurons in the spinal cord and brainstem was seen. In some of remaining motonerons, aggregates immunoreactive of misfolded SOD1 were found in the cytoplasm. Glial cells showed intranuclear reactivity for SOD1 using antibodies directed against misfolded SOD1.

Degeneration of the corticospinal tract and dorsal column was seen, as well as gliosis in the cortical areas of the frontal and temporal lobes and in the insula. Interestingly, these areas showed microvacuolar degeneration of the superficial lamina, especially in layer II and III.

Discussion: Positron emission tomography (PET) studies on D90A patients using [^{11}C]flumazenil binding has revealed changes both in motor areas as well as in non-motor areas such as the left fronto-temporal and anterior cingulated cortices. Our present finding of pathological microvacuolar degeneration in the superficial layers of temporal and frontal cortices supports in the FTD patient support this notion.

Interestingly in this pedigree both TDP-43 and SOD1 inclusions have been found, findings that some authors have regarded as mutually exclusive.

Conclusion: Pathological changes in the ALS patients showed for ALS characteristic changes and the FTD patient show frontotemporal pathology ssociated with FTD.

P168 OPTINEURIN PATHOLOGY IN ALS, FTLD-TDP, ALZHEIMER'S DISEASE AND HUNTINGTON'S DISEASE

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Keywords: immunohistochemistry, Optineurin (OPTN), Western blotting

Optineurin (OPTN) is a multifunctional protein involved in vesicular trafficking, signal transduction and gene expression. OPTN mutations were described in eight Japanese patients with familial and sporadic amyotrophic lateral sclerosis (FALS, SALS) (1). OPTN-positive inclusions co-localising with TDP-43 were described in SALS and in FALS with SOD-1 mutations, potentially linking two pathologically distinct pathways of motor neuron degeneration. We have explored the abundance of OPTN inclusions using a range of antibodies in post-mortem tissues from more than 100 cases and controls including sporadic and familial ALS, frontotemporal lobar degeneration (FTLD) and a wide range of neurodegenerative proteinopathies (2). OPTN-positive inclusions were detected in 34% of TDP-43-positive SALS spinal cord and 33% of FTLD-TDP. Western blot of lysates from FTLD-TDP frontal cortex and TDP-43-positive SALS spinal cord revealed decreased levels of OPTN protein compared to controls ($p < 0.05$), however this correlated with decreased neuronal numbers in the brain. Large OPTN inclusions were not detected in FALS with SOD-1 and FUS-mutation, respectively, or in FTLD-FUS cases. OPTN-positive inclusions were identified in a few Alzheimer's disease (AD) cases. Occasional striatal neurons contained granular OPTN immunopositivity in Huntington's disease (HD) but were absent in spinocerebellar ataxia type 3. No OPTN inclusions were detected in FTLD-tau and alpha-synucleinopathy. We conclude that OPTN inclusions are relatively rare and largely restricted to a minority of TDP-43 positive ALS and FTLD-TDP cases. Our results indicate a role of OPTN in neurodegeneration, however do not support the proposition that OPTN inclusions are crucial in the pathogenesis of ALS, FTLD or other neurodegenerative disorders.

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PI69 CLINICAL FEATURES AND PATHOLOGY OF AUTOSOMAL RECESSIVE AMYOTROPHIC LATERAL SCLEROSIS ASSOCIATED WITH OPTINEURIN MUTATION (P.Q398X)

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Keywords: OPTN, autosomal recessive, pathology

Background: *OPTN* is a causative gene for autosomal dominant, primary open angle glaucoma. We recently found autosomal dominant and recessive forms of *optineurin* (*OPTN*) mutations in patients with ALS.

Objective: We report the clinical features and pathology of ALS patients with the recessive *OPTN* mutation.

Methods: Clinical data was collected from two patients with the recessive *OPTN* mutation (p.Q398X). We conducted an autopsy in one patient. Brain and spinal tissue was studied with routine histological examination and immunohistochemical examination.

Result: Case 1: A 54-year-old Japanese woman presented with progressive swallowing difficulty and slurred speech. She was diagnosed bulbar type ALS. She died of respiratory failure at age 61. Duration of symptoms was 7 years. Case 2: A 44-year-old Japanese woman presented with progressive weakness of the right upper limb. She was diagnosed limb type ALS. She died at age 48 of respiratory failure. Duration of symptoms was 4 years. We detected a homozygous Q398X nonsense mutation in the *OPTN* gene in both patients. Autopsy was performed in case 1. In brain, macroscopic examination showed severe atrophy of bilateral motor cortex. Microscopically, severe loss of Betz cell and pyramidal neurons with gliosis were observed. Neuronal loss in the hypoglossal nucleus and facial motor nucleus was moderate. In spinal cord, macroscopic examination showed bilateral atrophy of the anterior root and the anterior horns of the spinal cord. Microscopically, moderate loss of anterior horn cell and gliosis were observed especially at the level of cervical and thoracic lesions. Bilateral corticospinal tracts were degenerated. Bunina bodies were not found. This case had degeneration in putamen, globus pallidus and substantia nigra. A few ballooned neurons and grain were found in amygdale and ambiens gyrus. OPTN immunohistochemical analysis did not show aggregation of OPTN.

Discussion and conclusions: The clinical phenotypes of these two patients with recessively inherited *OPTN* mutations were different in the sites of onset and disease courses. Because both patients have the same mutation and share a haplotype for 0.9Mb on chromosome 10 containing *OPTN*, these clinical differences may be derived from other factors. *OPTN* mutations may augment activation of nuclear factor-κB (NF-κB), which regulates genes involved at multiple stages of immune responses. The clinical course and phenotype of *OPTN* ALS may be influenced by environmental factors, including inflammation or other genetic factors affecting differences in inflammation sensitivity. The clinical features of autosomal recessive ALS patients with *OPTN* mutation appear to be indistinguishable from those of other sporadic ALS patients. Although the prevalence of pathological OPTN deletion and mutations are low, their phenotype similar to that in sporadic cases suggest that inappropriate activation of NF-κB may have a pivotal role in ALS in general.

P170 CHRONOLOGICAL SHIFT IN NEUROPATHOLOGICAL FINDINGS OF PATIENTS WITH ALS IN WAKAYAMA PREFECTURE ON THE KII PENINSULA

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Keywords: Kii-ALS, senile plaque, neurofibrillary tangle

Background: The reported incidence of ALS is quite high in the southern part of Wakayama (W) Prefecture on the Kii Peninsula, Japan, including Koza/Kozagawa/Kushimoto (K) area as well as in Guam. Although the incidence of ALS in these areas has gradually decreased since the 1980s, a relatively high frequency of ALS has persisted in K area in contrast to its disappearance on Guam. In the 1950s and 1960s, the neuropathological hallmark of ALS both on Guam and on the Kii Peninsula was the classical ALS pathology with neurofibrillary tangles (NFT) but without senile plaque (SP). Although the profile of cases on Guam changed to that showing SP in the 1990s, limited information is available regarding the profile of ALS cases on the Kii Peninsula.

Objectives: To clarify whether there have been any clinical and/or pathological changes in the profile of recent ALS cases on the Kii Peninsula.

Methods: In K area, samples from seven ALS patients who died before 1980 (Group I, mean 51.9 years old) and from three patients who died after 2000 (Group II, mean 72.3 years old) were re-examined. Six-μm paraffin sections of cerebral cortices, Ammon's horn, brainstem and spinal cord were stained using anti-PHF, anti-ß-amyloid, anti-human amyloidß, anti-TDP-43 polyclonal and monoclonal antibodies. Immunohistochemical examinations were performed using the ABC system and visualized with 3,3'-diaminoventidine. The appearance and distribution of NFT and SP as well as TDP-43 positivity were compared between Groups I and II.

Results: Clinical findings in these ALS patients showed upper and lower motor neuron signs without either overt dementia or parkinsonism and these findings did not differ between Groups I and II. Moderate amounts of NFTs were found in Ammon's horn and, to a lesser degree, in the cerebral cortex and brainstem in six cases of Group I (Braak stage III/IV). There was no apparent SP in cases of Group I, except for one patient who showed mild diffuse plaques. In Group II, all cases showed SPs (CERAD Criteria B) and NFTs in Ammon's horn and the cerebral cortex (Braak stage III/IV), but the

distribution and frequency of NFT were less prominent than those in Group I. In both Groups I and II, some spinal motor neurons showed TDP-43-positive cytoplasmic inclusions.

Discussion and conclusions: The appearance of NFTs in the cerebrum and TDP-43-positive inclusions in the spinal cord were characteristic findings of ALS in K area on the Kii Peninsula both before the 1980s and after 2000. The appearance of SPs was a characteristic of recent ALS cases in K area and might be, in part, related to aging. The unique tau-, TDP-43 and ß-amyloid pathology might be combined in ALS cases in the focus area on the Kii Peninsula.

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THEME 10 GENETICS

P171 ISOLATION AND IDENTIFICATION OF RNA TARGETS OF TDP-43 IN MOUSE BRAIN

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Keywords: TDP-43, genomics, gene regulation

Background and objectives: *TARDBP* gene mutations have been reported in both familial and sporadic ALS patients (1). TDP-43, a 43-KDa protein that binds to $(TG)_n$ repeats in DNA and corresponding $(UG)_n$ repeats in RNA, is encoded by the *TARDBP* gene. TDP-43 shuttles between the nucleus and cytoplasm and transports RNA (2). ALS patients without *TARDBP* mutations also display aggregation of TDP-43 suggesting that TDP-43 is important in the pathogenesis of ALS. The main objective of this work is to identify RNA targets of TDP-43 and to determine how these targets are misregulated in the presence of *TARDBP* mutations. This will help elucidate the underlying molecular mechanisms including the specific roles of TDP-43 in human disease and also help in developing better diagnostic and therapeutic opportunities. RNA targets of TDP-43 are candidate genes for searching for mutations in ALS patients where the cause remains unknown.

Methods and results: Combining both RNA-immunoprecipitation and microarray techniques (RIP-Chip), we identified more than 1000 potential RNA targets that bind to TDP-43 in mouse brain. A motif based sequence search using MEME suite revealed that the top TDP-43 targets identified by microarray contained $(TG)_n$ repeats corresponding to (UG) repeats in RNA. Reverse transcription polymerase chain reaction (RT-PCR) was then used to validate some of the binding targets identified using RIP-Chip. The RNA targets identified included the RNA binding protein gene, *Celf4,* the calcium/calmodulin dependent protein kinaseII alpha, *Camk2a, Septin 3* and the glial high affinity glutamate transporter, *Slc1a3.* Interestingly, our experiments on brain tissue from mice suggest a possible role for TDP-43 in regulating genes involved in synaptic transmission such as synapsin II *(Syn2)* and synaptophysin (*Syp*). Immunohistochemistry results show that TDP-43 is localized in the neuromuscular junction of phrenic-diaphragm motor neuron in mouse.

Discussion and conclusions: It has been suggested that RNA transport for site-specific translation in neurons is probably essential for synaptic transmission (3). Our RIP-Chip results from mouse brain suggest that TDP-43 plays a role in the regulation/transport of mRNAs involved in synaptic plasticity and that any dysfunction of TDP-43 might result in disruption of synaptic transmission.

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P172 ALTERED TDP-43 EXPRESSION IN LYMPHOCYTES FROM DEFINITE AND PROBABLE ALS PATIENTS WITHOUT TARDBP MUTATIONS

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Keywords: TDP-43, expression, PBMCs

Background: Amyotrophic lateral sclerosis (ALS) is an adult-onset progressive lethal motor neuron disease. TDP-43 is a ubiquitous multifunctional protein encoded by *TARDBP* involved in RNA processing. TDP-43, normally expressed in nuclei, has been shown to accumulate within pathological ubiquitinated cytoplasmic inclusions in brain and spinal cord of sporadic ALS patients and non-SOD1 familial ALS patients. In addition, previous Western blot analyses have shown that mutations in *TARDBP* produce TDP-43 aggregates in lymphocytes of ALS patients.

Objectives: Our objective was to determine the expression of TDP-43 in peripheral blood mononuclear cells (PBMCs) (*i.e.* lymphocytes and monocytes) in definite and probable ALS patients compared to healthy controls by immunocytochemistry.

Methods: Immunocytochemistry (ICC) experiments were performed using Shandon Cytospin^{TM} centrifugation and fluorescence microscopy on Histopaque^{TM} gradient-isolated PBMCs. The study group consisted of 9 definite ALS patients, 9 probable ALS patients, and 5 healthy control subjects. TDP-43 expression in lymphocytes and monocytes was determined by densitometry using ImageJ program. Confocal microscopy was used to determine cytoplasmic localization of TDP-43.

Results: Immunostaining of TDP-43 in monocytes was not significantly different between ALS patients and healthy controls. However, the relative expression of TDP-43 in lymphocytes *versus* monocytes (*i.e.* expression ratio) was increased in definite ALS patients compared to healthy controls ($p < 0.05$). In addition, the mean relative expression of TDP-43 in lymphocytes from probable ALS patients was intermediate compared to the mean relative expression of TDP-43 in lymphocytes from definite ALS patients and healthy controls. Cytoplasmic localization of TDP-43 in PBMCs was mostly detected in ALS patients with higher levels of TDP-43 expression and was not detected in PBMCs from healthy controls. No correlation was found between the expression of TDP-43 and the duration of

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the disease or the revised ALS functional rating scale (ALSFRS-R).

Discussion and conclusions: Increased relative expression of TDP-43 in lymphocytes of definite ALS patients compared to healthy controls is shown by immunohistochemistry for the first time in this study. The results support a role for TDP-43 in a systemic disease response without any association with the duration of the disease or the ALSFRS-R. TDP-43 expression alone is unlikely to represent a useful blood biomarker. However, the detection of a particular misfolded/unfolded form or abnormal translocation of TDP-43 in lymphocytes might still be indicative of disease-onset, severity or rate of progression.

P173 PHENOTYPIC HETEROGENEITY IN A LARGE CLUSTER OF SARDINIAN ALS CASES CARRYING A FOUNDER TARDBP A382T MISSENSE MUTATION

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Keywords: TARDBP, founder mutation, clinical heterogeneity

Background: Genetic studies of population isolates, such as Sardinia, are a powerful method to understand disease pathogenesis.

Objective: We undertook mutational screening of the *SOD1*, *FUS* and *TARDBP* genes in 197 patients of Sardinian ancestry diagnosed with ALS.

Methods: All patients underwent mutational analysis for *SOD1, FUS* and *TARDBP*. Specifically, all the coding exons and 50bp of the flanking intron-exon boundaries of *SOD1*, *FUS* and *TARDBP* were PCR amplified, sequenced using the Big-Dye Terminator v3.1 sequencing kit (Applied Biosystems Inc.), and run on an ABIPrism 3100Avant genetic analyzer.

Results: We found a c.1144G > A (p.A382T) missense mutation of the *TARDBP* gene in a quarter of cases (n = 46, 23.4%). The mutation was identified in 17 (39.5%) out of 43 fALS and 29 (18.8%) out of 154 apparently sALS. Considering 15 healthy carriers of the mutation, we calculated that the penetrance of the p.A382T mutation in the Sardinian population was 79% (95% c.i. 56-93) at 80 years. Patients carrying the A382T missense mutation had a better prognosis than patients who did not carry the mutation (3-year survival rate, 80.7% vs. 63.9%, p = 0.03). The presence of the A382T mutation remained independently significant also in Cox multivariable analysis.

The mutated cases carried a 94-SNP (663Kb) risk haplotype across the *TARDBP* locus on chromosome 1p36.22, indicating they shared a common ancestor. Flail arm and pyramidal phenotypes were significantly more frequent in patients carrying the p.A382T mutation than in those not carrying this mutation. Mutated patients were more frequently affected by frontotemporal lobar dementia (p = 0.02). Three of these patients developed extrapyramidal symptoms several years after their initial presentation with motor weakness.

Conclusions: We found that the *TARDBP* p.A382T missense mutation accounts for approximately one fourth of all ALS cases in this island population. These ALS patients share a large risk haplotype across the *TARDBP* locus indicating that they have a common ancestor. The phenotype of the mutated patients is quite heterogeneous, indicating that, even in presence of a founder mutation, other factors, genetics or environmental in nature, play a role in determining the ALS phenotype. Sardinian patients carrying the *TARDBP* p.A382T missense mutation have a better prognosis than patients who do not carry this mutation. The identification of mutated cases with extrapyramidal features expand the clinical spectrum associated with mutations in the TARDBP gene to include basal ganglia dysfunction.

P174 SCREENING FOR TARDBP MUTATIONS IN CHINESE SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: TARDBP gene, Chinese sporadic, high-resolution melting

Objective: Sporadic Amyotrophic Lateral Sclerosis (ALS) accounts for about 90% of patients. TDP-43 protein is coded by Transactive response DNA binding protein (TARDBP) gene and abnormal TDP-43 protein inclusion has been found in ALS patients. Many recent studies found TARDBP gene mutations in familiar and sporadic ALS. Our study is to screen TARDBP gene mutation in Chinese sporadic ALS patients.

Methods: We recruited 137 cases of sporadic ALS and extracted genomic DNA from blood samples. The coding region of TARDBP exon 6, was amplified by polymerase chain reaction (PCR). The PCR products were genotyped using high-resolution melting technology (HRM) and some of them were sequenced.

Results: We observed one heterozygous missense mutation (p.Gly348Val, c.G1043T) in one Chinese individual and one silent mutation (1098C > G) in two Chinese individuals with sporadic ALS. The mutation was not reported before. No mutation was found in 90 control Chinese individuals.

Conclusions: One heterozygous missense mutation and one silent mutation in TARDBP was found in 137 Chinese cases. Our data indicates that genetic variation in TARDBP (0.73%) may not be a common cause of sporadic ALS in Chinese population.

P175 NINE PEDIGREES WITH MUTATIONS IN FUS/TLS IN FAMILIAL ALS CONTAINING A NEW TRUNCATING MUTATION

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Keywords: FUS, genetics, familial

Background: Mutations in the *FUS/TLS* gene have been associated with familial amyotrophic lateral sclerosis (fALS).

Methods: We analyzed the presence and frequency of C-terminal *FUS/TLS* mutations in a German ALS cohort, including 184 fALS and 247 sALS patients by sequence analysis of exons 5, 6, 13 -15.

Results: We identified nine pedigrees containing seven heterozygous *FUS/TLS* mutations and two truncating mutation in German ALS families, including the R521H, K510R, R514G, R495X and G478X. The truncating mutation were both associated with an aggressive disease course whereas the R521H, K510R and R514G mutation showed a mild phenotype with disease duration ranging from 6 to 8 years.

Conclusions: Mutations in *FUS/TLS* account for 8.7 % (16 of 184) of fALS in our German cohort.

P176 MUTATIONAL ANALYSIS OF THE FUS/TLS GENE IN A CATALAN ALS POPULATION

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Keywords: FUS/TLS, epidemiology, MIM 608030

Background: Previous epidemiological studies suggest that mutations in the FUS/TLS gene are the second most common genetic cause of familial ALS (FALS). A predominantly autosomal dominance inheritance pattern was observed in these pedigrees harboring the FUS/TLS mutational variants, and in a few FALS patients with an autosomal recessive inheritance pattern. Sporadic ALS (SALS) cases have occasionally been described.

Genetic characterization of ALS6 families should provide information on the distribution of FUS/TLS mutations in different ethnic groups. The prevalence of FUS/TLS gene mutations in Catalonia, a Spanish region of 7,000,000 inhabitants, has not been studied.

Objective: To determine the prevalence of FUS/TLS gene mutations in a Catalan ALS population, and to analyze the genotype-phenotype relationship.

Materials and methods: 30 different FALS pedigrees and 124 sporadic ALS patients were screened for FUS/TLS gene mutations by direct sequence analysis using the methodology previously described.

Results: Clinical information about the disease's characteristics was available for 120 individuals in the FALS group (65 males and 55 females), with a male-female ratio of 1.18:1. The disease began with limb onset in 62.5% of cases, bulbar onset in 11.7%, and simultaneous bulbar and limb onset in 12.5%. No clear data concerning the exact site of onset was available in the remaining patients (13.3%). The mean age at onset was 49.3 years old (S.D. 12.0; median 50.0; range 21-81 years). A FUS/TLS mutant was identified in two of the 30 FALS pedigrees studied. The mutations found in this group were p.R521C and p.K510E. The phenotype in the p.R521C family was characterized by a young age at onset (38.2 years old), proximal limb girdle weakness, predominant lower motor neuron signs and dropped head. Survival time ranged from 10 to 36 months. Obligate asymptomatic carriers were detected. The phenotype in the p.K510E family was of an early onset (<40 years old), predominant lower motor neuron disease with survival of less than one year.

The mutational variant p.R522R was identified in a 74-year-old woman with bulbar onset in the sporadic ALS (SALS) group. The prevalence of FUS/TLS gene mutations in our FALS population was 6.6%, while in the SALS group it was 0.8%.

Conclusions: The prevalence of FUS/TLS mutations in FALS in Catalonia (6.6%) is similar to levels in other European populations, making ALS6 the second most common form of FALS in our population. In the SALS group, our results (0.8%) are similar to other epidemiological studies.

The high percentages of FUS/TLS mutations in other FALS populations and the presence of obligated asymptomatic carriers increases the probability that other genetic factors are involved in this disease's pathogenesis/epidemiology.

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P177 FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS WITH GLY93SER MUTATION IN CU/ZN SUPEROXIDE DISMUTASE: A CLINICAL AND NEUROPATHOLOGICAL STUDY

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Keywords: Gly93Ser mutation, SOD-1, posterior column

Background: Familial amyotrophic lateral sclerosis (FALS) with abnormalities of the Cu/Zn superoxide dismutase (SOD-1) gene is neuropathologically characterized by the degeneration of middle root zones of the posterior column and the presence of Lewy body-like hyaline inclusions (LBHIs) in the lower motor neurons, in addition to the involvement of the upper and lower motor neurons. We report here an autopsy case of FALS with Gly93Ser missence mutation in exon 4 of the SOD1 gene in which no clinical or detailed pathological data have been available.

Methods: The patient's aunt died of ALS at age 67. Her first symptom was weakness of the leg muscles. Weakness of the hand muscle was noted 1 year after the onset of the disease. Our patient developed insidious muscle weakness in the legs, and her gait gradually became disturbed from age 23. At age

24, she also experienced weakness in the arms. At age 30 she exhibited hoarseness due to bilateral cord palsy. At the same age, she presented with dysphasia. She became bedridden from the age of 36 years. About 16 years after onset of the disease, at the age of 39 years, the patient died of respiratory failure. She did not show dementia, opthalmolegia, or autonomic disturbances. During the course of her illness, deep tendon reflexes were decreased without pathological reflexes.

Results: Histological examination disclosed conspicuous changes throughout the spinal cord. In the anterior horns from cervical to lumbar segments, there was marked loss of neurons with gliosis, and there was absence of neuronal inclusion bodies in the remaining cells. Neither Bunina bodies nor LBHIs were present in the neurons. In the medulla oblongata, the hypoglossal nuclei showed mild loss and shrinkage of nerve cells. Marked atrophy and myelin pallor were present in the superior cerebellar peduncles. The dentate nuclei of the cerebellum showed neuronal loss accompanied by gliosis and grumose degeneration. The precentral gyrus showed mild loss and shrinkage of Betz cells. There was a moderate neuronal loss with gliosis in the red nucleus. The corticospinal tracts in both anterior and lateral funiculi showed a slight degree of myelin pallor. There were circumscribed areas of myelin loss in the posterior column from the cervical through the lumbar segments. There was also a marked myelin loss in the spinocerebellar tract. Prominent neuronal loss was noted in the Clarke's nuclei.

Discussion: The clinical characteristics in this case was onset of symptoms in the legs, frequently seen in FALS, and slow progression: 16 years survival. Based on clinical, genetic and pathological findings with a review of the literature, we suggest that degeneration of the dentatorubral system and the absence of LBHIs in our case are pathological features in FALS with the Gly93Ser mutation.

P178 A CASE OF ALS4 WITH A NOVEL SENATAXIN GENE R2136C MUTATION WITH PARTIALLY IMPROVED WEAKNESS AND SENSORY DISTURBANCE OF LIMBS BY IMMUNOTHERAPY

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Keywords: senataxin, ALS4, immunotherapy

Background: Mutations in the senataxin gene (SETX) were reported in patients with two distinct sydromes, familial amyotrophic lateral sclerosis (ALS) 4 and ataxia oculomotor apraxia type 2. Only three senataxin mutations, T3I, L389S, T1118I and R2136H, have been reported in ALS4 patients.

Objectives: The present study aimed to clarify clinical and pathological features of familial ALS4 with partially improved weakness and sensory disturbance of limbs by immunotherapy.

Methods: We carried out clinical, neuropathological, and genetic studies on a Japanese patient with the new senataxin gene (R2136C) mutation.

Results: The patient, a 41-year-old male, was born in normal delivery and had a tendency to fall in childhood. His feet showed pes cavus since childhood. He suffered from progressive weakness of lower limbs and gait disturbance from age 35. Within 1 year from onset, bilateral arm weakness developed and progressively worsened, and sensory and urinary disturbance also occurred. On first admission to our hospital at 37-year-old, he was diagnosed to have chronic inflammatory demyelinative polyneuropathy. Methylpredonisolone pulse therapy improved weakness of four limbs. The steroid pulse therapy was repeated at the time of exacerbations of weakness and sensory disturbance of limbs. Neurological examination at 41-year-old revealed a distal dominant severe weakness, atrophy and fasciculation in four limb muscles. Hyperreflexia was also noted in four limbs. The planter response was extensor type bilaterally. Sensory impairment of the lower limbs and urinary disturbance were noted. Neither tongue atrophy nor dysarthria was observed. A nerve conduction study revealed sensorimotor peripheral neuropathy; the conduction velocities of motor and sensory nerves and the amplitudes of compound muscle action potentials were severely reduced in all four limbs. Cervical MRI showed thickening and enhancement of nerve roots. Sural nerve biopsy showed a slight decrease in large myelinated fibers and endoneurial infiltration with lymphoid cells. Although the missense mutation R2136C was found in exon 17 of SETX in the patient, his genetically related parents and a sister did not have the mutation, suggesting the proband carried a *de novo* mutation.

Discussion and conclusions: This case showed distinct clinical features, as compared with previously reported cases, such a hypertrophy of nerve roots on MRI, inflammation of the biopsied sural nerve, and favorable response to immunotherapy. This case suggests that an immunological mechanism may be partly involved in SETX mutation.

P179 CLINICAL-GENETIC CHARACTERIZATION OF THE FIRST TWO SPANISH FAMILIES WITH SPASTIC PARAPLEGIA HARBORING A 6-EXON DELETION (EX10-16 DELETION) IN THE SPG4/SPAST GENE

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Keywords: hereditary spastic paraplegia, SPG4, clinical-genetic relationships

Objectives: Hereditary spastic paraplegia (HSP) is a clinically and genetically heterogeneous group in which at least 45 different loci have been identified. The inheritance pattern may be AD, AR, or linked to the X chromosome. The most frequent form of AD-HSP is that caused by mutations in the SPG4/SPAST gene. Its prevalence is 15-40%, depending on the population studied. Most mutations in this gene are "missense" and "non-sense" although "splice-site" mutations and

small deletions/insertions have been described. However, large deletions are very unusual. Here we present the first two SPG4/SPAST Spanish families carrying a deletion of exons 10-16.

Patients and methods: We studied the phenotype, age of clinical onset, age of wheelchair dependence, neurophysiological examinations, and the Spastic Paraplegia Rating Scale (GeNeMove) scores in 10 members of two non-interrelated *AD-HSP* families. We carried out direct sequencing of the genes *SPAST, ATL1, REEP1* and *NIPA1* and the MLPA assay with the Salsa Kit P165 (MRC Holland) of the SPAST gene under standard conditions in 6 patients.

Results: The average age of clinical onset was 29.1 years old (range 6-50). All patients presented all the clinical features characteristic of pure forms, although five referred fecal incontinence as one of the initial symptoms, which was extremely debilitating in two. The average age of loss of gait autonomy and wheelchair confinement was 57.7 years old. The mean score on the GeNeMove scale was 22.3. The Barthel score was 68.6. In one of the families, we observed an intergenerational variability in the age of onset, suggesting a phenomenon of anticipation. TMS highlighted different degrees of conduction defects. In both families, we identified a deletion that covered exons 10-16 (c.1227-1729 + ?) of the SPAST gene that was confirmed using RNA.

Conclusions: This is the first description of a large *SPG4/SPAST* deletion in the Spanish population. The presence of fecal incontinence as one of the initial symptoms could be interpreted as a distinctive clinical finding for this mutation as it has not been described in the phenotype of other deletions described to date. Anticipation and almost complete penetrance were other characteristics associated with this deletion.

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P180 AN AUTOSOMAL DOMINANT FAMILIAL MOTOR NEURON DISEASE WITH A NOVEL MUTATION OF A GENE ASSOCIATED WITH NF-KB SIGNALLING PATHWAY

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Keywords: NF-kB, HMSNP, p65

We found a novel mutation of a gene in two families with motor neuron disease previously reported as HMSNP. The disease typically starts at 35-62 years of age in proximal muscles with prominent fasciculations, and progresses over 5-20 years symmmetrically affecting distal muscles as well. Later loss of vibratory sense becomes apprant at the feet on examination, but never presents sensory disabilities. Most patients die of respiratory failure or pneumonia. Autopsy findings included both upper and lower motor neuron invlovement where abnormal accumulation of the gene product was found. Immunohistochemical analysis of NF-kB p65 subunit showed its localization in the nucleus of neurons in the patient, whereas only cytoplasm was stained in normal spinal motor neurons. Mutant gene was found to affect NF-kB reporter expression in HEK293T cells on relative luciferease assay.

P181 GENE EXPRESSION ANALYSIS IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS AND MULTIFOCAL MOTOR NEUROPATHY

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Keywords: mRNA, biomarker, microarray

Background: There are no reliable biologic markers for amyotrophic lateral sclerosis (ALS). Multifocal motor neuropathy (MMN) without conduction block can mimic ALS. Thus, a method to separate patients with ALS and MMN is needed.

Objectives: To confirm the previously identified gene expression pattern in muscles from patients with ALS and MMN compared to controls.

Methods: RNA extracted from skeletal muscles of 3-ALS, 3-MMN and 3-control patients were subjected to RT-PCR confirmation analysis based on our previously published genome-wide microarray gene expression data (1). Four additional ALS patients and four new controls were also used.

Results: Validation analysis of the most significant expression pattern differences confirmed our previous microarray data for leucine-rich repeat kinase-2 (LRRK-2), follistatin, collagen type XIX alpha-1, ceramide kinase-like, sestrin-3 which were overexpressed only in the ALS group. CXorf64 was increased in ALS and decreased in MMN compared to controls. Western blot analysis of LRRK-2 gene protein level from ALS muscles showed no obvious difference compared to controls.

Discussion and conclusions: The up-regulation of the above genes in the muscles of ALS patients relative to MMN and controls discovered previously by our microarray analysis is reproducible and statistically significant. Further studies are necessary to evaluate the identified genes in larger patient groups and different tissues.

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P182 ATAXIN-2 INTERMEDIATE-LENGTH POLYGLUTAMINE: A POSSIBLE RISK FACTOR FOR CHINESE PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: Ataxin-2 (ATXN2) gene, polyglutamine (polyQ) expansion

Background: Intermediate-length polyglutamine (polyQ) expansions in the Ataxin-2 (ATXN2) gene have recently been identified as a risk factor for sporadic amyotrophic lateral sclerosis (SALS). Our study aims to analyze (CAG)n expansions in the ATXN2 gene among Chinese patients with SALS.

Methods: All patients diagnosed with adult-onset sporadic ALS were consecutively followed up, and their clinical characteristics were collected. To measure the repeats length of ATXN2 polyQ, fluorescence-polymerase chain reaction products were analyzed on a 3100-Avant Genetic Analyzer Applied Biosystem using the ROC-500 size standard.

Results: Three hundred and forty-five patients with SALS were studied. The mean age of onset was 51.38 ± 12.45 years. ATXN2 polyQ with a repeat length greater than 27 was found to be weakly associated with ALS in our study. There was no significant difference in mean age of onset, gender, and onset site between the group of SALS patients with and without ATXN2 polyQ expansion greater than 27.

Conclusion: Our finding provides evidence that the ATXN2 polyQ expansion greater than 27 might be a risk factor for Chinese SALS patients.

P183 ASSOCIATION STUDY OF SPORADIC AMYOTROPHIC LATERAL SCLEROSIS IN CHINESE PATIENTS BY USE OF MALDI-TOF MASS SPECTROMETRY

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Keywords: case-control association analysis, single nucleotide polymorphism, MALDI-TOF genotyping

Objective: Sporadic amyotrophic lateral sclerosis (ALS) accounts for majority of patients. ALS is a kind of complex disorder. There were several SNPs reported to be associated with sporadic ALS in recently published genome-wide association studies (GWAS), but there are few data from Asian ALS populations and no report focuses on single nucleotide polymorphisms which may be associated with sporadic ALS of Chinese origin.

Methods: We have recruited 86 individuals with sporadic ALS and 94 matched controls for our study and extracted genomic DNA from blood samples. Genotypes were determined by a MALDI-TOF based approach followed by association analysis.

Results: Individual genotype data for 8 SNPs in Chinese population showed no significant association with sporadic ALS. Combining genotype data with published GWA, rs1942239 gained in strength of allelic association, and rs558889 deviated Hardy-Weinberg equilibrium at ALS case group which may be associated with susceptibility to sporadic ALS.

Conclusions: SNP rs1942239 and rs558889 may contribute to susceptibility of sporadic ALS in Chinese patients. The larger sample studies are warranted to confirm the association.

P184 FINDING THOSE ELUSIVE GENETIC VARIANTS IN SPORADIC ALS: ARE WE LOOKING AT THE RIGHT TISSUE?

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Keywords: somatic mutation, copy number variant, genome array

Background: The majority of analyses of blood DNA looking for a genetic basis for SALS have been unsuccessful. These include investigations of common genetic variants that could lead to disease susceptibility, or of rare variants that could be directly mutagenic. This lack of success may be because some SALS-causing mutations occur predominantly in brain tissue, and are either absent or are at presently undetectable levels in blood.

Objective: In an attempt to gauge the extent of genetic variability between blood and brain tissue in SALS, we looked for differences in genome-wide chromosomal copy number variants (CNVs) between these two tissues in SALS patients.

Methods: The 32 SALS patients were 22 male and 10 female Australians. Blood samples were taken during life, and patients also gave consent for post mortem removal of brain and spinal cord tissue. Genomic DNA was extracted from peripheral blood nucleated cells and from the cortex of the lateral frontal gyrus from frozen brain tissue. Genome-wide CNVs were compared between blood and brain from the SALS patients, as well as in 26 control brains (to exclude common brain-only polymorphisms) using Affymetrix 6.0 (1.8 million marker) arrays and Partek software.

Results: 410 CNVs were detected that were present in SALS brain but not blood DNA. These involved 94% of the SALS patients, with a median of 8 brain-CNVs per patient. Twenty-four of the brain-CNVs were rare, were not found in control brains, and overlapped with genes. Some of these genes are involved in processes suspected in SALS, e.g. apoptosis, glutamate metabolism, and RNA editing. Ten SALS brain-CNVs were found in more than one patient.

Discussion: Brain-predominant structural variants may be common and appear to be present in most patients with SALS. Many of these are likely to be brain-predominant private polymorphisms, and any mutagenicity of these variants remains to be tested.

Conclusion: Although with this number of subjects this needs to be regarded as a preliminary study further multi-tissue studies of this nature, with larger numbers of samples and using more sensitive methods of copy number detection, are warranted to look for brain-specific mutations that could underlie SALS.

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THEME 11 BMAA

P185 SAMPLE MATRIX INTERFERENCES IN ANALYTICAL METHODS FOR DETECTION AND QUANTIFICATION OF BMAA

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BMAA is a non-protein amino acid found in samples of cyanobacteria, traditional foods and people of Guam. In *in vitro* studies, BMAA has been shown to be neurotoxic leading to interest in the prevalence of the amino acid in other parts of the world. However, difficulties in the measurement of BMAA in complex biological matrices have resulted in conflicting reports. Mass spectrometry (MS) is often seen as the "gold standard" of analytical techniques and analysis of BMAA by the fragmentation of the parent as m/z 119 -> 102 has been reported. This method of detection is prone to false negative error caused by chromatography interferences, voltage fluctuations, ion suppression and sample matrix effects. Only 0% - 28% of underivatized BMAA standard eluted from C18 columns using the published columns and eluents. MS issues were identified using a Time of Flight MS with mass accuracy 0.001. Optimal detection of the parent mass 119.028 is achieved at low inlet voltages (<30) and MS response declined dramatically at higher inlet voltages to less than 20% of the standard. Regardless of voltages, all BMAA solutions tested contain a 102.055 signal at a steady 26% of the total ion count and corresponding to the daughter ion indicating spontaneous loss of the OH group in solution. Depending on voltages uses, between 3% and 66% of the BMAA standard was detected as the compound dimer with m/z 237.156. Interestingly, higher voltages increased the concentration of the dimer rather than increasing the daughter ion signal. Further, the measurement of BMAA in marine cyanobacteria is complicated by the formation of ion adducts in salt water. BMAA standards made in salt water solutions contain m/z signals of 143.525 (Mg-BMAA; 38%), 142.21 (Na-BMAA; 12%) and 159.183 (Ca-BMAA; 9%). Together, ion adducts account for more than 50% of the BMAA ion signal in underivatized standards. In contrast, ACQ-derivatized BMAA (m/z 459.17) forms a Na adduct (m/z 481.162) in marine samples that accounts for less than 4% of the total ion signal. These data indicate that, while MS can be a useful tool for analysis of BMAA, sample matrices and instrumental factors can lead to lost signal and false negative results.

P186 BMAA IS A CYANOBACTERIAL METABOLITE

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Background: β-N-methylamino-L-alanine (BMAA) has been associated with certain forms of progressive neurodegenerative disease, including sporadic Amyotrophic Lateral Sclerosis and Alzheimer's disease. The source of BMAA considered to have contributed to ALS/PDC on Guam was initially identified as *Cycas micronesica*. Subsequently, symbiotic *Nostoc* in the corraloid roots of cycads were shown to contain BMAA. Neurotoxic, non-protein amino acids have been intensively studied in eukaryotic phototrophs but have received less attention in cyanobacteria although BMAA in cyanobacterial cultures, and in cyanobacterial blooms continues to be reported by investigators from a variety of laboratories. However, it was recently suggested, based on reported instances of failure to detect BMAA in cyanobacteria, that BMAA may be confused with other compounds during analysis or that cyanobacteria may not be the source of the toxin.

Objectives: We sought to confirm the analytical validity of BMAA identification methods and to apply these methods to confirm the cyanobacterial origin of BMAA by stable isotope feeding of axenic and uni-algal cyanobacterial cultures. We also investigated the effect of biologically available nitrogen on cellular BMAA content so as to address the wide range of BMAA content observed in cyanobacteria. Additionally, we sought to confirm the presence of BMAA in organisms exposed to cyanobacterial blooms to support our data on uptake of BMAA by a range of organisms.

Methods: Two pre-column derivatization methods with either LC/MS or LC/MS/MS detection were used to distinguished BMAA from related compounds and to quantify BMAA in BMAA-exposed and unexposed samples. Nitrogen modulation of cyanobacterial culture media and ^{15}N feeding experiments were used to evaluate BMAA production in axenic and uni-algal cultures, and the rate of assimilated nitrogen incorporation into BMAA.

Results: BMAA production by cyanobacteria was confirmed in axenic and uni-algal cultures of cyanobacteria based on chromatrographic retention time and by mass spectrometry using parent ion and product ions and ion ratios resulting from collision-induced dissociation. Nitrogen starvation of nutritionally replete cells resulted in an increase in free cellular BMAA. The addition of NO_3^- and NH_4^+ to the culture medium following starvation resulted in a decrease of free cellular BMAA, with ammonia resulting in a more rapid reduction. Addition of ^{15}N ammonium chloride resulted in

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the appearance of ^{15}N-containing amino acids, with no significant increase in ^{15}N-containing BMAA. Subsequent starvation resulted in the appearance of ^{15}N-containing BMAA.

Discussion and conclusion: BMAA is clearly distinguishable from common isomers and diamino acids in cyanobacteria. The production of BMAA by an axenic culture of cyanobacteria confirms the cyanobacterial origin of BMAA. This is further supported by the production of isotopically labeled BMAA by axenic cyanobacterial cultures from supplied raw materials, and by the presence of BMAA in aquatic organisms exposed to cyanobacterial blooms.

P187 CYANOBACTERIAL BLOOMS AND THE OCCURRENCE OF THE NEUROTOXIN BMAA IN FLORIDA AQUATIC FOOD WEBS

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Background: Recent papers have suggested that virtually all cyanobacteria species produce the neurotoxin beta-N-methylamino-L-alanine (BMAA). A large increase in the development of Amyotrophic Lateral Sclerosis (ALS)-Parkinsonism dementia complex in Guam in the middle of the 20th century has led to the hypothesis that the neurotoxin BMAA may be involved. High concentrations of BMAA have been found in humans who died of ALS or Alzheimer's disease, but little or no BMAA in the brains of those who died of other causes. These results suggest that BMAA from cyanobacteria could be involved in the development of neurodegenerative diseases.

Objectives: To determine if BMAA can biomagnify in aquatic food chains, BMAA concentrations were analyzed in animals collected from water bodies known to have blooms of cyanobacteria, primarily in Florida.

Methods: Cyanobacteria abundance was estimated by measuring concentrations of phycocyanin with a spectrofluorometer. BMAA was quantified by detection of the AQC fluorescent tag detected by reverse phase HPLC and verified by LC/MS/MS using product ion mode in a triple quadrupole system.

Results: Samples of pink shrimp in Florida Bay where a large bloom of cyanobacteria developed in central Florida Bay in the 1980s and persists have high concentrations of BMAA. Samples of fish and crustaceans in South Biscayne Bay-Eastern Florida Bay where a large bloom of cyanobacteria developed for 2 years show some species with no BMAA and others with very high concentrations. High concentrations were found in fish in the Caloosahatchee River where large blooms of cyanobacteria develop as a result of nutrient rich water from the Everglades Agricultural Area. High concentrations of BMAA were found in fish and blue crabs in Chesapeake Bay where blooms of cyanobacteria occur. Concentrations of BMAA similar to that of ALS and Alzheimer's patients were found in the brain tissue of bottlenose dolphins in the Indian River Lagoon where large blooms of cyanobacteria occur.

Discussion: These data suggest that BMAA could be found in high concentrations in aquatic animals in many areas of the world where cyanobacteria blooms occur.

Conclusions: A wide range of concentrations (ranging from undetectable to approximately 7000 ug/g) of the neurotoxin BMAA were found in various animals, from crustaceans to dolphin brains, in aquatic food webs. This suggests that BMAA can biomagnify up the food chain from cyanobacteria to seafood.

P188 IDENTIFYING ENVIRONMENTAL TRIGGERS OF AMYOTROPHIC LATERAL SCLEROSIS IN NORTHERN NEW ENGLAND

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Keywords: BMAA, cyanobacteria, epidemiology

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease. Sporadic ALS (sALS) represents the vast majority of cases, while approximately 5% of cases are familial and inherited (fALS). sALS likely results from a combination of genetic and environmental risk factors, however, few environmental factors have been identified as possible triggers for the disease. One proposed mechanism is exposure to the cyanobacteria produced neurotoxin β-methylamino-L-alanine (BMAA), which has been implicated as a cause of ALS/Parkinsons-Dementia Complex in Guam. Recent studies supporting this hypothesis found high levels of BMAA in the brain tissue of ALS patients and demonstrated a mechanism for bioaccumulation of BMAA within the food chain on Guam. Because cyanobacteria are found ubiquitously throughout the world, from the Baltic Sea to the desert sands of Iraq, our research aims to identify routes of exposure to BMAA and identify clusters of sALS in Northern New England, USA. Using 2010 Census and Landscan USA data, we constructed an expected model of sALS cases in Vermont, New Hampshire and Maine based on population density, age, and gender. Using geo-spatial analysis tools in ArcGIS we identified areas where the observed cases of ALS significantly exceeded the predicted number. A number of these clusters are located on water bodies known to harbor cyanobacterial blooms, potentially linking them to BMAA. In order to further examine the etiology of ALS and gather specific home address data for the previous analysis, an in-depth questionnaire was administered to over one-hundred and fifty ALS patients as well as to controls throughout the region. The questionnaire explored possible relationships between ALS and previously reported risk factors including smoking, military service, as well as potential exposure to BMAA which is produced by cyanobacteria found in blue-green algal blooms on lakes and rivers throughout New England. Sediment core, water, and bloom sample analysis has allowed for the detection of BMAA and historical identification of cyanobacteria in New England water bodies. Further geo-spatial analysis through the expansion of our study population will allow us to better determine if a correlation exists between proximity and exposure to cyanobacterial blooms and the development and onset of ALS.

P189 PROTEINS CONTAINING BMAA FORM AUTOFLUORESCENT AGGREGATES AND INDUCE CELL DEATH

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Keywords: BMAA, aggregation, apoptosis

Background: β-methylamino-L-alanine (L-BMAA), a non-protein amino acid produced by cyanobacteria (blue-green algae), has been linked to a neurodegenerative disease in the South Pacific (ALS/PDC) and more recently to sporadic Amyotrophic Lateral Sclerosis (sALS). Cyanobacteria are ubiquitously distributed in terrestrial, fresh water and marine environments thus human exposure to L-BMAA, possibly via bioconcentration through the food chain, is a likely route. Critically, the vast majority (>90%) of L-BMAA present in extracts of cyanobacteria, cycad seeds, brain tissue from Chamorro ALS/PDC patients and from North American ALS and Alzheimer's patients is in a 'protein-associated form'. Here we extend these studies to examine the toxicity of BMAA-containing proteins.

Objectives: To determine if proteins containing incorporated BMAA are toxic to cells.

Methods: Human fibroblasts (MRC-5) and neuroblastoma cells (SH-SY5Y) were incubated in amino acid-free media, supplemented with 500 μM L-BMAA in the presence and absence of the protein synthesis inhibitor cycloheximide (CHX) for 24 hrs. Cells were then examined by inverted fluorescent microscopy – for autofluorescent aggregates. Cell viability was assessed using acridine orange (AO) to indicate apoptosis and ethidium bromide (EtBr) to indicate necrosis.

Results: In cultures incubated with L-BMAA, perinuclear autofluorescence was observed under fluorescent light. Cells incubated with CHX (2 μg/mL) alone remained viable, while cells incubated with 500 μM BMAA showed an ~4-fold increase in apoptosis/necrosis. In the presence of CHX, which reduces BMAA incorporation into protein by 75%, apoptosis/necrosis was significantly reduced. Further, co-incubation with amino acids to "out-compete" BMAA incorporation, also protected the cells from death.

Discussion: Post-mitotic cells, in particular neurons, are extremely sensitive to misfolded proteins, possibly because these potentially toxic species cannot be diluted out of the cell by normal cell division. As such, cellular inclusion bodies and aggregated mutant proteins, such as TDP-43 and alpha synuclein, have been implicated in the etiology of ALS. We have previously shown that the misincorporation of non-protein amino acids results in the formation of autofluorescent aggregates and cell death via apoptosis (1).

Conclusions: In cell culture, incubation with L-BMAA resulted in the formation of autoflourescent aggregates and the induction of apoptosis and necrosis. Thus, chronic exposure to BMAA may eventually result in the accumulation of misfolded/aggregated proteins which may induce cell death and thus, contribute to the pathology of sALS.

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P190 EFFECTS OF THE CYANOBACTERIAL TOXIN, -METHYL-AMINO-L-ALANINE (BMAA), ON HUMAN NEURONS AND RAT GLIAL CELLS

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Keywords: BMAA, primary human neurons, glia

Background: The amino acid variant β-methyl-amino-L-alanine (BMAA) has long been associated with the increased incidence and progression of the amyotrophic lateral sclerosis/Parkinson's disease complex (ALS/PDC) within the Chamorro people from Guam (1). BMAA has been reported to be produced by more than 90% of cyanobacterial species (2), and is therefore potentially ubiquitous in the environment. Even though BMAA has been detected in the brains of Chamorro people who died of ALS/PDC (3), as well as in the post-mortem brains of Alzheimer's disease (4) and ALS sufferers in North America, its effects on human primary neurons or neuron associated glial cells have not been determined.

Objectives: Here, we have challenged human primary neurons (HPN) and rat olfactory ensheathing cells (OECs) with exogenous BMAA, at concentrations from 100 μM to 1 mM, and measured its effects on cellular toxicity, apoptosis and generation of reactive oxygen species (ROS).

Methods: OECs and HPN were exposed to pure BMAA and media assayed for LDH release. DNA damage, generation of ROS and Ca^{2+} influx were measured. Neuronal nitric oxide synthase (nNOS) and caspase 3 cleavage were monitored microscopically in HPN. Mitochondrial activity of OECs was also measured. RNA was extracted from OECs and subjected to microarray analysis.

Results: We show that BMAA is cytotoxic, increases Ca^{2+} influx, enhances production of ROS and causes DNA damage in both cell types. Furthermore, BMAA disrupted mitochondrial activity in OECs. Caspase 3 cleavage and expression of nNOS were observed. Microarray expression data suggested a role of BMAA in the induction of apoptosis.

Discussion and conclusion: The results indicate that both cell types are significantly compromised after treatment and that the cyanobacterial toxin acts by direct excitotoxicity and via disturbance of mitochondrial activity. This is the first study investigating BMAA toxicity using human primary neurons and pure glial cells. The gliotoxicity of BMAA highlights its role as a key player in neurodegenerative disease as it can disturb neuronal homeostasis at several levels. The data presented align BMAA with the three proposed mechanisms of degeneration in ALS, those being non-cell autonomous death (5), excitotoxicity and mitochondrial dysfunction (6). The cytotoxicity of BMAA on different brain cells has important implications for the aetiology, progression and treatment of neurodegenerative disease.

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P191 SYNERGISTIC TOXICITY OF THE ENVIRONMENTAL NEUROTOXINS METHYLMERCURY AND BETA-N-METHYLAMINO-L-ALANINE (BMAA)

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Keywords: BMAA, methylmercury, glutathione

Background: Determination of the environmental factors involved in ALS has been elusive. Methylmercury and β-N-methylamino-L-alanine (BMAA) have both been implicated in this role. However, studying these factors in isolation probably does not accurately mimic the human condition. ALS often likely involves a complex interaction between genetic predisposition and multiple environmental factors. As a first step to assess how such factors may interact, we studied the interaction of BMAA and methylmercury. BMAA and methylmercury are widespread in the environment and exposure to both is likely common occurrence.

Objectives: The objective of the study was to test whether there is a synergistic effect between methylmercury and BMAA in causing neurotoxicity and then to determine the mechanism of the synergistic toxicity.

Methods: We used primary mixed neuronal and glial cortical cultures from embryonic mice to study the toxicity of BMAA and methylmercury. Neuronal death was assessed by release of the cytosolic enzyme lactate dehydrogenase. Since high concentrations of both methylmercury and BMAA have been shown to deplete cellular glutathione levels, we chose to analyze glutathione as a potential point of interaction between the two toxins. Cellular glutathione levels were assessed using an enzymatic assay that measures total glutathione.

Results: Exposure of cultures to methylmercury or BMAA independently induced concentration dependent neurotoxicity. The death caused by each toxin was selective to the neurons. Importantly, concentrations of BMAA (10 - 100 mM) that caused no toxicity by themselves potentiated methylmercury (3 mM) toxicity. BMAA plus methylmercury, at concentrations that had no effect by themselves on glutathione levels, together induced depletion of cellular glutathione. Furthermore, the combined toxicity of methylmercury and BMAA was attenuated by the cell permanent form of glutathione, glutathione monoethyl ester or the free radical scavenger, trolox. The combined toxicity was not blocked by the NMDA receptor antagonist MK-801. Toxicity of a high concentration of BMAA is mediated primarily by NMDA receptor activation, with significant protection provided by MK-801, and protection by trolox only occurring when NMDA receptors are blocked. Neurotoxicity of high concentration methylmercury in this culture system is attenuated by glutathione monoethyl ester, but not by trolox or MK-801. Therefore, the combined toxicity is somewhat different than that of each toxin alone. There is clearly a prominent role of oxidative stress in the neurotoxicity of the combined toxin treatment.

Discussion: There are two main findings of the current study. First, there is a synergistic toxicity induced by exposure to a combination of methylmercury and BMAA. Second, the mechanism of the synergistic toxicity appears to be at the level of cellular glutathione depletion.

Conclusions: Since glutathione depletion is known to occur in ALS, these results provide a potential mechanism for the involvement of methylmercury and BMAA in ALS.

P192 DISEASE PROGRESSION WITH EARLY TOXIN-INDUCED NEUROPATHOLOGY IN THE AGEING MUTANT SOD MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: superoxide dismutase (SOD), motor axon, glia

Background: Familial adult onset amyotrophic lateral sclerosis (FALS) poses injuries to the central nervous system that lead to progressively debilitating and irreversible motor deficits. The loss of motor neurons results from a poorly understood, multifactorial neurodegenerative process. Mutations in the gene encoding superoxide dismutase 1 (SOD1) are one cause of FALS. In contrast, sporadic ALS occurs with a vastly greater incidence and from unknown etiologies. Among suspected causes are various environmental toxins. An early report of environmental causes of neurodegenerative disease, including a form of ALS, pointed to a long latency neurotoxin in cycad seeds amongst the Chomorro people of Guam. Both washed cycad as well as isolated water insoluble steryl glucosides (SG) similar to that found in cycad seeds reproduced an ALS-PDC phenotype in an *in vivo* model.

Objectives: To determine whether environmental agents such as those from cycad accelerate disease onset in an otherwise late-onset condition, we combined two *in vivo* models of ALS testing dietary SGs for their potential synergistic properties in combination with genetic predisposition to adult onset ALS in the G37R mouse.

Methods: Male and female mice were treated with 42 mg of SG per kilogram of body weight daily in their diet. A cohort of animals harboured the SOD1G37R mutation for genetic predisposition to ALS.

Results: Results showed an additive effect of SG on spinal motor neuron loss and caused decreases in average soma diameter. While the presence of the transgene alone caused a leftward shift towards smaller diameter ventral root axons, SG exposure alone resulted in a bimodal distribution resembling a more immature state. The presence of the transgene alone markedly increased the amount of GFAP- and Iba1-positive cells in the spinal cord grey matter, with a heterogeneous expression of ramified (resting) and activated morphologies. The transgene in combination with SG did not significantly change glial numbers, but caused all glial cells to become extensively activated.

Discussion and conclusions: Although the mechanism of cycad toxin-induced neurodegeneration remains uncertain, the current results showed that dietary exposure to SGs alone was sufficient to produce a disease phenotype, but that when implemented in conjunction to a genetic predisposition to ALS was sufficient to produce a more severe disease phenotype. In conclusion, the environmental agent studied here has direct cytotoxic effects and contributes to disease progression

in ALS. The mouse model of disease exploited in this study may be used further to understand the mechanisms of motor neuron death and CNS pathology in degenerative conditions exacerbated by environmental agents.

P193 CONCURRENT ELECTROPHYSIOLOGICAL AND LOCOMOTOR DEFECTS IN A FED DROSOPHILA MELANOGASTER MODEL OF ALS-PDC

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Keywords: BMAA, environment, electrophysiology

Background: The L-form of the non-natural amino acid, beta-methylamino alanine (L-BMAA) is implicated in the ALS variant disease called amyotrophic lateral sclerosis-Parkinsonism dementia complex (ALS-PDC). While genetics and age can be effectors of MND, environmental factors like BMAA, produced by several or more species of cyanobacteria, contribute to neuronal dysfunction by affecting the glutamate NMDA and AMPA receptors. *Drosophila melanogaster* (fruit fly) is an example of an animal model that can be used to study the phenotypic effects of BMAA and the equivalent *in-vivo* effects at the neuronal level; glutamate is the major neutrotransmitter for fruit flies.

Objectives: The development of a fruit fly model to quantitatively characterize the locomotor defects of fed-flies, while simultaneously recording the dose-dependent and temporal development of defects at the post-synaptic neurons of flight muscles, using electrophysiology (EP).

Methods: The negative geotaxis or tapdown test was conducted to characterize gross locomotor defects over a period of 3-5 days continuous feeding of 12.5, 25 and 50 mM L-BMAA. Intracellular electrophysiological recordings were measured in individual Canton S flies that were anesthetized and immobilized, followed by insertion of the recording and stimulus electrodes for EP measurements. Flies were stimulated at pulses of 10 to 40 mHz. In all cases recording was done from the dorsal longitudinal flight muscles. This non-invasive method allows recording of the same fly over a period of several days.

Results: Three days after acute BMAA exposure, fed-flies showed a dose-dependent loss of locomotor climbing ability. The average percent of flies able to reach the top of the vial after tap down was 9.0% (50 mM), 33.6% (25 mM), 78.3% (12.5 mM) and 91.0% for the control female fly. The viability was also dose-dependent. Viability improved significantly with lysine and alanine; glutamate or histidine gave moderate protection when each amino acid was co-fed with equimolar BMAA. The EP showed spontaneous responses, decreases in the responses to low-level stimulation (1-10 Hz) and the inability to follow trains of stimulation at the postsynaptic DLM. These defects were progressive from days 1 to 3 during the feeding period. BMAA (4 mM) directly applied to the fly showed the same response effects and exogenous glutamate applied post-BMAA attenuated BMAA. Day 1 EP recordings showed defects at the DLM, prior to any significant locomotor disabilities.

Discussion and conclusions: The dose-dependent loss of motor ability correlates with the equivalent EP loss at the post-synapse level indicating BMAA acts at the neuronal level. The competition experiment with the chemically similar alanine and glutamate (equivalent to carbamylated-BMAA) showed a rescue in the viability and leads to developing a mechanism of where and how BMAA acts on the NMDA and AMPA glutamate fruit fly receptors.

P194 ZINC INHIBITION OF BMAA TOXICITY

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Background: Previous studies into the Guamian ALS-Parkinson's Dementia complex have identified β-methylamino-L-alanine (BMAA), as the potential neurotoxin responsible for this disease. BMAA is a non-essential amino acid produced by cyanobacteria, that are present in all ecosystems. The hypothesis has been that some individuals are vulnerable to BMAA deposition into their central nervous system where it is incorporated into proteins which can then serve as a reservoir for this neurotoxin. If BMAA is a potential neurotoxin responsible for ALS then methods directed at decreasing this toxin could treat the disease. It has been shown that BMAA binds strong to transition metal ions such as zinc, which may prevent BMAA from crossing into the brain and possibly clear BMAA from the CNS. In addition, zinc may serve as an antioxidant in the CNS and help protect the Blood Brain Barrier against oxidative stress and prevent BMAA from crossing into the brain. We hypothesize that by exposing patients to high levels of zinc, BMAA would be kept in a bound complex with zinc, which would increase its clearance.

Objective: To determine the safety and tolerability of zinc methionine (Optizinc) at high doses in patients with sporadic ALS and to measure levels of BMAA in blood and urine pre and post treatment.

Methods: Ten patients diagnosed with sporadic ALS on stable doses of riluzole were enrolled. Patients received Optizinc at 30 mg TID and copper 2 mg QD (to prevent copper depletion) for three months. Blood and urine were collected and shipped to the Institute for Ethnomedicine for BMAA analysis at baseline and at month 3. ALS-FRS-R, safety labs, and zinc and copper plasma levels were measured monthly. FVC and Quality of Life visual analogue scale (QOLVAS) were measured at baseline and at month 3.

Results: Eight patients completed the study. One patient went into a hospice and dropped out after one month of treatment. One patient was hospitalized for pneumonia early in the study. The majority of patients tolerated Optizinc at 90 mg/d. Only one patient couldn't tolerate the dose due to nausea, but completed the study on 60mg/d. Zinc levels were maintained between 80% and 125% of the upper limits of normal. Copper levels were maintained within the normal range in all patients. The rate of ALS progression, as measured by monthly change in ALS-FRS-R scores, did not improve or worsen in any of the patients over the course of treatment. All patients who completed the study (N = 8) opted to stay on treatment. Levels of BMAA from the blood and urine are being analyzed and will be presented.

Conclusion: Optizinc at 90mg/d was well tolerated in patients with ALS. No effect on the rate of disease progression was observed.