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SESSION 1 JOINT OPENING SESSION

WELCOME

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COMMUNICATING SCIENCE TO POLITICIANS AND THE PUBLIC

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SESSION 2A CELL BIOLOGY & PATHOLOGY

C1 THE BLOOD-BRAIN BARRIER AND ITS RELEVANCE TO PATHOGENESIS AND THERAPY IN NEURODEGENERATIVE DISEASE

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Keywords: blood brain barrier; blood-spinal cord barrier

The blood-brain barrier (BBB) is formed by the endothelial cells lining the cerebral microvessels; the blood-spinal cord barrier (BSCB) is the equivalent structure in the spinal cord. The chief characteristic of these barriers is the extreme tightness of the zipper-like 'tight junctions' that link neighbouring endothelial cells, making the vessels in the central nervous system (CNS) around 50–100 times tighter than those of peripheral tissues such as muscle and skin.

The tight barrier significantly reduces the ability of water soluble compounds to diffuse into or out of the brain via the junctional cleft (paracellular pathway), so that most molecular traffic across the endothelium is via the cells (transcellular pathway). Small gaseous molecules such as oxygen and carbon dioxide can diffuse freely through the cell membranes, as can lipid soluble agents such as ethanol and many CNS drugs. Water-soluble molecules the brain needs such as glucose and amino acids can be carried across the endothelium on specific 'transporters', and efflux transporters help exclude or get rid of toxic agents and metabolites, but also exclude some potentially useful CNS drugs.

The barriers are critical to normal healthy function of the CNS, especially by maintaining a constant central environment for neuronal activity. The perivascular space around the microvessels bounded by the endfeet of astrocytic glia has a further protective role, by providing a 'niche' for elements of the cellular immune system.

Disturbances in CNS barrier function are present in a number of neurodegenerative disorders (NDDs), including multiple sclerosis, Alzheimer's disease and stroke; they can contribute to exacerbation of symptoms and neural damage, and may also play a causative role under some conditions. There is growing recognition that CNS inflammation is an element in many of these disorders, with the BBB, BSCB and perivascular niche being critical sites for the inflammatory process.

The evidence for BBB/BSCB involvement in ALS is relatively new, from observations in patients, and from animal models especially the G93A mutation of superoxide dismutase SOD1. Changes reported include leakage of plasma proteins into the CNS and cerebrospinal fluid (CSF), compromised endothelial tight junctions, and changes in vascular anatomy and blood flow. Thus for ALS, as for many other NDDs, the endothelium of CNS microvessels may be a promising target for drug therapies, to maintain endothelial and barrier health, and to repair the dysfunctional barrier.

More research is needed, sampling CSF and plasma to follow the course of barrier disturbance in human disease, animal experimentation to reveal features of the barriers in vivo, and cell culture models to allow detailed studies of the mechanisms of action at the cellular and molecular level. The CNS endothelium may prove a useful future target for ALS therapy.

C2 A NEW ROLE FOR ANGIOGENIN IN NEURITE PATHFINDING AND SURVIVAL-IMPLICATIONS FOR ALS

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Keywords: Angiogenin, pluripotent stem cells, neuroprotection

Introduction: Amyotrophic lateral sclerosis (ALS) is a late onset neurodegenerative disorder affecting upper and lower motor neurons (MNs) with fatal consequences. The molecular mechanisms underlying ALS are poorly understood and mutations in SOD1 is one of the known causes of ALS. However, mutations in SOD1 are seen only in a very small number of cases of ALS. Interestingly, mutations in human angiogenin (hANG), a member of the ribonuclease A (RNase A) superfamily known to be involved in neovascularization, have been recently reported in patients with ALS (1–4) but the expression and function of Ang in the nervous system and the effects of these mutations on MN differentiation and survival has not been investigated.

Objectives: The main objectives are 1) to study the expression of Ang during mouse embryogenesis 2) to investigate its importance in neuronal differentiation 3) to study the effects of the hAng-ALS variants on neurons.

Methods: The distribution of Ang in the mouse developing nervous system was investigated by immuno-histochemistry. The pluripotent embryonal carcinoma cell culture model of neuroectodermal differentiation was used to study the expression of Ang during MN differentiation by immunocytochemistry and effect of a small molecule inhibitor of Ang on neuronal differentiation was investigated. The hANG-ALS variants were generated by site directed mutagenesis and the proteins were expressed and purified. The effects of hANG and hAng-ALS variants on MN differentiation, neurite extension as well as their ability to protect MNs from hypoxia

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induced cell death was investigated using the cell culture model.

Results: Mouse angiogenin-1 (mAng-1) is strongly expressed in the developing nervous system in mouse embryogenesis and during neural differentiation of pluripotent P19 embryonal carcinoma cells. NCI 65828, a cell permeable inhibitor of hANG inhibits neurite extension/pathfinding by P19 derived neurons without affecting their differentiation to the neuronal lineage (5). We also report that neurite extension/pathfinding by pluripotent EC cell derived neurons treated with the hANG ALS mutants is compromised. hANG ALS mutants also have a cytotoxic effect on MNs leading to their degeneration. hANG was able to protect neurons from hypoxia induced cell death but the mutants of hANG lacked neuroprotective activity.

Discussion and Conclusions: Our findings show that ANG plays an important role in neurite pathfinding and survival providing the first causal link between mutations in hANG and ALS.

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C3 RETINOID SIGNALING ALTERATIONS IN ALS AND THE CONSEQUENCES OF THESE ALTERATIONS IN MOTOR NEURON-ENRICHED CULTURES

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Keywords: retinoid signaling, RARbeta, motor neurons

Background: Previous reports demonstrated decreased transthyretin (TTR) levels in the cerebrospinal fluid of ALS patients. TTR plays multiple physiological roles and functions within the central nervous system to regulate protein aggregation, antioxidant activity and the retinoid signaling pathway. Multiple studies have described the differential expression of genes regulated by retinoid signaling in post-mortem tissues from individuals with ALS. Similarly, alterations in retinoid-regulated gene expression have been observed in transgenic animal models of ALS prior to disease-related morphological changes and symptom onset.

Objectives: Our overall objectives were to identify and then explore the functional consequences of alterations of the retinoid signaling pathway in ALS to test the hypothesis that retinoid signaling is deficient in ALS and that it contributes to the motor neuron cell death that occurs in the disease.

Methods: Using post-mortem lumbar spinal cord tissue, we characterized the expression and distribution of retinoid signaling components with immunohistochemistry, immunoblotting and confocal microscopy. An *in vitro* motor neuron-enriched cell culture system was then used to perform more functional studies in which we focused on the nuclear genomic and non-genomic effects of retinoic acid signaling in motor neurons.

Results: Differences in cytoplasmic binding proteins including cellular retinol binding protein (CRBP) and cellular retinoic acid binding proteins (CRABP)-I and II were observed. CRBP immunoreactivity was higher in ALS spinal cord motor neurons while CRABP-I was decreased. CRABP–II was concentrated to the nucleus in ALS motor neurons while control motor neurons exhibited a diffuse cytoplasmic pattern. We also assessed the expression and distribution of retinoic acid nuclear receptors. RARβ exhibited increased immunoreactivity in motor neuron nuclei of individuals with sporadic ALS although both controls and individuals with familial ALS lacked this punctate nuclear immunostaining. In our *in vitro* system, we modulated individual nuclear receptors with agonists and antagonists to further characterize their effects on gene transcription and simultaneously evaluated the more rapid, non-genomic effects by assessing phosphatidylinositol-3-kinase (PI3K) and ERK1/2 MAPK signaling pathways.

Conclusions: Our results indicate that retinoic acid signaling is altered in ALS and components of this pathway may represent novel therapeutic targets. In particular, the differences we have observed with respect to RARβ suggest that retinoid signaling impacts only the sporadic form of the disease while it does not play a role in familial cases. This may indicate differences between the two forms of the disease.

C4 METABOLIC DIFFERENCES BETWEEN BRAIN AND SPINAL CORD MITOCHONDRIA OF WILD TYPE AND HUMAN FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS MUTANT SOD1-TRANSGENIC RATS

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Keywords: mitochondria, calcium, ROS, metabolism

Background: ALS is associated with hypermetabolism and mitochondrial dysfunction (1, 2). In SOD1 during the early pre-symptomatic stage, many spinal cord mitochondria (SCM) in large myelinated axons exhibit swelling with an increased number of cristae, and show small vacuoles in the matrix, cristae or both (3). With neuronal activation, brain mitochondria (BM) and SCM utilize a mixture of substrates: glutamate, pyruvate and malate, with increased ATP production. However, in resting BM and SCM, oxidation of glutamate, pyruvate and malate increased ROS generation due to increased reverse electron transport (RET).

Objectives: To determine, quantitatively with our novel methodology, differences between BM and SCM from wild type (WT) and SOD1 rats in major mitochondrial functions: oxidative phosphorylation, permeability transition and mechanisms of ROS generation.

Methods: Non-synaptic BM and SCM from 8 week old WT and SOD1 rats were analyzed for tissue Ca^{2+} content by atomic absorption, respiration by Mitocell S200 Micro Respirometry System and ROS by Amplex Red.

Results: In comparison with WT, in SOD1 the yields of BM were diminished by 20% and SCM by 57%. The respiratory activities were also significantly diminished with all substrate mixtures, particularly in SOD1 SCM. The Ca^{2+} content in the WT spinal cord was 8 fold larger than in WT brain. The calcium retention capacity (CRC) of BM from WT exceeded 4-fold the total Ca^{2+} content in WT brain (both per 1g of wet tissue). The CRC of WT SCM corresponded to only 10% of the total WT spinal cord Ca^{2+} content. Both WT and SOD1 SCM showed much higher rates of succinate oxidation than

WT and SOD1 BM. Despite significantly diminished rates of state 4 respiration, SCM and BM from SOD1 rats showed several-fold higher rates of ROS generation with glutamate, pyruvate and malate which was sensitive to malonate.

Conclusions: SCM have significantly lower intrinsic inhibition of succinate oxidation, which resulted in higher rates of the RET-driven ROS generation as compared with BM from both WT and particularly SOD1 rats. Loss of mitochondria in spinal cord of SOD1 rats begins long before neurological dysfunction. These data suggest that poorly buffered Ca^{2+} released during the pre-symptomatic phase of neuroaxonal degeneration, together with increased ROS generation may be responsible for early loss of SCM in SOD1 rats.

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C5 DIFFERENTIALLY EXPRESSED BIOLOGICAL PROCESSES IN RELEVANT SPINAL COMPARTMENTS ISOLATED BY MICRODISSECTION IN SOD1 TRANSGENIC MICE

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Keywords: Genomics, gene expression, microarray

Background: There are now three published whole genome expression profiles of laser captured motor neurons. We performed similar studies except that we also: (a) profiled anterior horns as well as motor neurons; (b) used 2 different controls; (c) looked at very early time points; and (d) analyzed biological processes as well as single genes.

Objectives: To profile early differentially expressed biological processes in the cellular compartments relevant to motor neuron degeneration in G93A mouse model of ALS.

Methods: We used G93A mice and 2 controls (G93A littermates and human wild type transgenic mice). We studied the mice at 20 and 60 days. We created 2 separate RNA pools, 1 enriched with motor neurons isolated by laser capture microdissection and 1 enriched with anterior horns collected after removal of motor neurons. We synthesized a cDNA probe using in vitro transcription amplification. We profiled gene expression using whole genome oligonucleotide microarray. We processed microarray data for biological enrichment using Gene Set Analysis (GSA) (www-stat.stanford.edu/ ~ tibs/GSA/), which profiles differentially expressed sets of genes representing biological processes defined in Gene Ontology rather than just differentially expressed individual genes.

Results: *In the motor neuron compartment at 20 days*: there was up-regulation of purine nucleoside monophosphate biology, negative regulation of B-cell activation, and thyroid hormone metabolism; there was down-regulation of adenylate cyclase activation, amino acid derivative metabolism, peptide hormone processing, and positive regulation of lyase activity. *At 60 days*: there was up-regulation of serine metabolism and DNA damage response, signal transduction resulting in induction of apoptosis; there was down-regulation of apoptotic nuclear changes, aspartate family amino acid metabolism, cholesterol/sterol biosynthesis, regulation of B cell proliferation, DNA catabolism, and G-M transition of mitotic cell cycle. *In the anterior horn compartment at 20 days*: there was up-regulation of glutamate metabolism, JAK-STAT cascade, regulation of amino acid metabolism, regulation of phosphate metabolism and regulation of phosphorylation, regulation of protein amino acid phosphorylation, and positive regulation of peptidyl-tyrosine phosphorylation; there was down-regulation of adenylate cyclase biology, regulation of lyase activity, phospholipid catabolism, peptide hormone processing, and mechanosensory behavior. *At 60 days*: there was up-regulation of carbohydrate biosynthesis and gluconeogenesis; there was down-regulation of negative regulation of Wnt receptor signalling pathway, neurogenesis, neuron differentiation, positive regulation of B cell proliferation, and positive regulation of JNK cascade.

Discussion and Conclusions: GSA allows the discovery-based power of microarray to be carried into data interpretation. The deregulated biological processes were distinctly resolved but very different between the motor neuron and anterior horn compartments, as stated above. The processes deregulated at 20 days did not remain deregulated at 60 days. There were marked differences just comparing the 2 different controls to each other and marked improvement in the signal resolution in disease by using both. Data interpretation still remains the biggest challenge for these studies.

C6 GENE EXPRESSION PROFILING TO INVESTIGATE THE STRESS EFFECTS OF PHYSICAL EXERCISE ON THE MOTOR NEURONE TRANSCRIPTOME

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Keywords: exercise, motor neurons, microarrays

Background: A body of evidence indicates that physical activity is implicated as a risk factor in ALS: 1. Well known sportsman such as Lou Gehrig (baseball) and Donald Levey (football) have developed MND; 2. In several studies, participation in sports/athleticism has been identified as a risk factor; 3. Recent reports have shown an increased incidence of ALS in Italian and American soccer players. The hypothesis has been put forward that a high level of physical exercise is associated with an increased risk of developing ALS in individuals with an underlying genetic susceptibility, which may result in a failure to mount the normal physiological response to physical exercise. We have investigated the transcriptome of motor neurones (MN), the vulnerable cell population in MND, and muscles in response to exercise.

Objective: Our aim is to identify 1) which genes are activated/repressed in response to exercise in muscles and MN in absence of other stress; 2) which genes belong to a specific motor neuronal response; 3) which cellular compartments are under stress during physical training and whether these can be involved in the pathophysiology of ALS.

Methods: Gastrocnemius muscle and approximately 1000 MN have been isolated from lumbar spinal cord of 3 female mice undergoing voluntary exercise (mean running distance of 13km per day) for 21 ± 1 days and 3 sedentary mice. Muscular RNA was isolated using Quiazol kit (Quiagen); motor neuronal RNA was extracted using Picopure kit (Arcturus), amplified using the Affymetrix Amplification kit (Arcturus) and labelled using the GeneChip Expression IVT Labelling Kit (Affymetrix). 10 μg cRNA was applied to the

Affy Mouse Genome 430 2.0 GeneChip, and data analysis was performed using ArrayAssist (Iobion).

Results: After 3 weeks of voluntary exercise, analysis of the motorneuronal transcriptome showed upregulation of 203 transcripts and downregulation of 241. The main changes affect genes encoding for neurotrophic factors and their receptors, i.e. ciliary neurotrophic factor, leukaemia inhibitor factor receptor and activin receptor 2a; genes involved in neurotransmitter release and regulation of ion currents and membrane potential, i.e. K^+ and Ca^{2+} channels, along with genes involved in the regulation of NMDA receptor expression at the synapse, i.e. the splicing factor Nova2. Mechanisms such as branching process and transcription modulation are also altered. In gastrocnemius muscle 194 genes were upregulated and 176 downregulated. The main changes affect angiogenesis, favoured by upregulation of VEGF receptor 2 and Epas1; myogenesis, stimulated by increased levels of Trkb and Hdgf and ECM reorganisation.

Conclusions: Our study highlights some important similarities between the physiological response of MN and skeletal muscle to exercise and the pathophysiology of ALS. 1. The key role of Cntf, Lif and Vegfr2. 2. The potential deregulation of K^+ channels as cause of the abnormal membrane electric properties affecting ALS patients and their balancing function in exercise. 3. Axonal growth and branching process, likely to be altered in ALS, are fundamental in the reinforcement of the NMJ in response to exercise. Functional polymorphism studies on candidate genes in ALS patients will be used to investigate the relation between the mechanisms altered in response to exercise and the pathophysiology of ALS.

C7 AN AMYOTROPHIC LATERAL SCLEROSIS-ASSOCIATED MUTATION IN VAPB IMPAIRS AXONAL TRANSPORT OF MITOCHONDRIA

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Keywords: Axonal transport, Mitochondria, VAPB

Background: A mutation in vesicle-associated membrane protein-associated protein B (VAPB; VAPBP56S) causes familial ALS but the mechanisms whereby VAPBP56S induces disease are not fully understood. We have previously shown that disruption to axonal transport is a very early event in mutant superoxide dismutase 1 (SOD1) induced ALS (1). The early disruption of axonal transport suggests that compromised axonal transport of selected cargoes may be causative rather than secondary in ALS.

Objectives: The objective of this study was to analyze the effect that VAPBP56S has on axonal transport of mitochondria.

Methods: Mouse cortical neurons were transfected with empty vector, wild-type VAPB or VAPBP56S and axonal transport of mitochondria labeled with mitochondria-targeted red fluorescent protein was quantified from time-lapse recordings as described previously (1).

Results: VAPBP56S significantly decreased the total number of motile mitochondria by specific reduction of the number of anterogradely-transported mitochondria; net retrograde transport of mitochondria was unaffected. This specific inhibition of net anterograde transport significantly shifted the balance of axonal transport of mitochondria. Detailed quantification of the mitochondrial transport activity showed that VAPBP56S induced a significant decrease in anterograde transport activity but did not affect retrograde transport activity.

The velocity of transport is an intrinsic property of the molecular motors that drive transport. Hence to assess any direct damage to molecular motors, the transport velocity was determined by tracking individual mitochondria through axons. VAPBP56S did not affect anterograde or retrograde transport velocity indicating that molecular motors were not damaged.

Conclusion: These observations suggest that VAPBP56S specifically inhibits anterograde transport of mitochondria by affecting the regulation of the anterograde motor kinesin-1. Interestingly, the nature of this impairment of anterograde transport and the resulting imbalance of mitochondrial transport is very similar to that caused by ALS mutant SOD1 (1).

Our findings provide new insights into the mechanisms by which VAPBP56S induces motor neuron demise. Furthermore, our analyses reveal that disturbance of anterograde mitochondrial transport is a common feature in at least 2 distinct forms of familial ALS. As such, correcting axonal transport defects represent a promising therapeutic target.

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C8 SPASTIN MUTATIONS DISRUPT AXONAL TRANSPORT IN HEREDITARY SPASTIC PARAPLEGIA (HSP)

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Keywords: spastin, axonal, transport

Background: Mutations in the spastin gene, on the SPG4 locus, are the most prevalent cause of HSP, accounting for approximately 40% of cases. Spastin is a microtubule severing protein, and several groups have demonstrated that both its knockdown and the overexpression of disease-causing mutants result in axonal defects. We hypothesise that spastin mutations directly disrupt axonal transport.

Objectives: To gain insight into how mutations in spastin lead to axonal degeneration in HSP by quantification of axonal transport of mitochondria in neurons in the presence or absence of mutant spastin.

Methods: Mice harbouring a point mutation in a splice donor site in the SPG4 gene were identified and re-derived onto a C57BL/6 background. Primary cortical neurons were prepared from E15.5 homozygous and heterozygous mutant spastin mice and wild-type littermate controls. Immunocytochemistry was performed on fixed cultures to examine the presence of axonal swellings, using antibodies raised against cytoskeletal and vesicular cargoes. Mouse cortical neurons were co-transfected with DsRed2Mito and EGFP-empty vector. Live cellular imaging was performed and various properties of mitochondrial motility were analysed. To determine the presence of axonal swellings in adult mice, immunohistochemistry was performed on cervical and lumbar spinal cord sections from homozygous and heterozygous mutant spastin mice of various ages and wild-type littermate

controls to identify axonal swellings. Similar axonal transport data were recorded in rat primary cortical neurons co-transfected with DsRed2Mito and either EGFP-spastin wild type, EGFP-spastin K388R, or EGFP empty vector.

Results: We show that the mouse spastin mutation effects mRNA splicing and causes the creation of a premature stop codon. Axonal swellings occur in cultured mutant spastin neurons. Swellings are associated with significant accumulations of a number of cytoskeletal and vesicular cargoes. These cargo accumulations were also identified in the corticospinal tract of adult mice. Specific properties of both anterograde and retrograde transport were disrupted in the presence of mutant spastin. We demonstrate that swellings are dynamic structures as mitochondria, although often stalled temporarily, were able to enter and leave these structures. In transfected rat neurons the K388R spastin mutant caused comparable defects in axonal transport.

Discussion: Together these results demonstrate that 2 different types of spastin mutation directly disrupt axonal transport, and are likely to play an important role in the dying back axonopathy, which is the principal pathological feature of HSP. As the axonal transport defects and the abnormal accumulation of cytoskeleton and vesicular cargoes are evident in the heterozygous mutant spastin mice, we promote the current spastin mouse model as an effective tool to study SPG4-linked HSP pathophysiology.

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SESSION 2B MND PHENOTYPES

C9 ALS MIMIC SYNDROMES

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Keywords: mimic ALS, diagnosis

The core clinical syndrome of Charcot amyotrophic lateral sclerosis is unmistakeable to clinical neurologists once the disease has evolved to encompass mixed upper and lower motor neuron signs with clear progression. However, despite evidence that the rate of diagnostic error is low in specialist settings, the grave implications of making the diagnosis of ALS still leads to considerable anxiety amongst both specialists and non-specialists. Diagnostic doubt can contribute to management delays, impede open communication between doctor and patient and promote excess investigation. Particular areas of difficulty arise in young (<40 years) people with ALS, atypical regional syndromes (flail limb, isolated corticobulbar palsy), very slowly progressive cases and the existence of an ALS-like paraneoplastic syndrome. Using a systematic approach based on mode of presentation, this talk will review conditions which can mimic ALS and suggest a practical approach to identifying which cases require more extensive investigation.

C10 NATURAL HISTORY AND PROGNOSIS OF THE FLAIL ARM AND FLAIL LEG SYNDROMES

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Keywords: Flail arm, Flail leg, Prognosis

Objective: We sought to define the clinical, prognostic and biological significance of the flail arm and flail leg variants of amyotrophic lateral sclerosis (ALS) in comparison with typical forms of sporadic ALS.

Methods: We analysed clinical phenotypes in relation to survival in two clinic cohorts at the King's MND Care and Research Centre London, UK and the Bethlehem MND service in Melbourne, Australia. Using a standard protocol we categorised patients into phenotypes including bulbar onset and classical limb onset ALS, the flail arm syndrome (FA) and the flail leg syndrome (FL). 1137 cases were analysed from the King's cohort and 432 from the Bethlehem sample. Survival times from disease onset were analysed using the Kaplan- Meier method and the Cox proportional hazards model. Demographic and clinical characteristics between groups were compared using Oneway ANOVA with Dunnett's t test for post hoc comparisons.

Results: In the King's cohort, median survival for limb onset ALS was 34 months and for bulbar onset ALS it was 27 months ($p < 0.001$). In contrast, median survival for the FA syndrome was 61 months ($p < 0.001$ compared to limb onset) and for the FL syndrome, 69 months ($p < 0.001$ compared to limb onset). Five year survival rates in the King's cohort were 9% for bulbar onset, 20% for limb onset, 52% for FA syndrome, and 64% for FL syndrome. In the Bethlehem cohort median survival for limb onset ALS 31 months, bulbar onset 27 months ($p = 0.001$ compared to limb onset), FA syndrome 66 months ($p < 0.001$ compared to limb onset) and the FL syndrome 71 months ($p = 0.001$ compared to limb onset). Five year survival rates were 9% for bulbar onset, 19% for limb onset, 52% for FA and 77% for FL variants. The ratio of men to women was 4:1 in the FA group compared to 1.5:1 in bulbar and limb onset cases. Age of onset was not significantly different between FA cases and limb onset ALS (Mean 57.3 years; $p = 0.89$) or FL cases and limb onset ALS (Mean 55 years; $p = 0.90$). 25% of flail arm cases and 33% of flail leg cases did not fall within the 2000 Revised El Escorial criteria. Median delay in diagnosis time was significantly longer in both flail arm (17 months; $p < 0.001$) and flail leg subgroups (25.5 months; $p < 0.001$) compared to limb onset ALS (11 months). Median time to spread to a second region was significantly longer in flail arm (29 months; $p < 0.001$) and flail leg cases (33 months; $p < 0.001$) in comparison to limb onset ALS (8 months). The median time from onset to NIV use was 22 months for limb onset ALS, and was significantly longer in the FA syndrome (median 51 months, $p < 0.001$) and the FL (median 52.5 months, $p < 0.001$).

Conclusions: Median and five year survival estimates for the FL and FA phenotypes were significantly longer than for more typical forms of ALS. These syndromes also show longer diagnostic delays, time to requiring ventilation remain localised for longer to the region of onset for longer than typical ALS. Our findings suggest that these syndromes have both clinical importance and biological relevance.

C11 THE CLINICAL FEATURES THAT DISTINGUISH PLS FROM ALS

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Keywords: primary lateral sclerosis, PLS, Diagnosis

Background: Previously we reported that patients with clinically pure PLS, with no evidence of LMN dysfunction on examination or EMG after 4 years, had a significantly better prognosis and better function than patients with ALS. Patients with "UMN-dominant ALS," defined by predominantly UMN signs and minor LMN signs, had findings and prognosis intermediately between PLS and typical ALS. Prior to the fourth year from symptom onset, the diagnosis of PLS could not be made reliably because many patients developed LMN signs before that time. Predicting which patients with solely UMN dysfunction are destined to develop LMN signs is difficult, but important to patients and to the design of clinical trials. We undertook the current project to determine how clinical features can be used to suggest a diagnostic category and provide an indication of eventual outcome. The specific objective was to analyze which examination findings help distinguish between categories at the first visit and during follow-up.

Methods: We reviewed the records of 21 patients (9 PLS and 13 UMN-dominant ALS) who had repeated follow-up for more than 4 years, the earliest time of reliable diagnosis, as well as those of 10 randomly selected control patients with ALS seen in 1984–2007. ANOVA F-tests for continuous variables and chi-square tests for categorical variables assessed differences in baseline data among the three diagnostic

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categories. Linear and logistic regression analyses with subject random effects assessed the relation between examination data over time and diagnostic group.

Results: At first examination, only the lowest score on the weakest muscle ($p < 0.001$) and the site of onset ($p = 0.041$) discriminated between eventual diagnostic group; PLS patients were stronger and more likely to have limb-onset than either the UMN-dominant or ALS groups. Across all visits, group assignment significantly predicted FVC ($p = 0.026$) and ALSFRS-R ($p = 0.009$) scores. UMN-dominant and ALS groups were more likely to have muscle atrophy ($p = 0.009$) or lost weight ($p = 0.004$), even when controlled for dysphagia ($p = 0.020$), and ALS patients were more likely to have hyporeflexia ($p = 0.001$) than the other groups. An MRC score of 4 or less on any muscle was associated with the diagnosis of ALS ($p = 0.0001$), but not PLS.

Conclusions: Features at baseline most suggestive of eventual LMN signs are muscle weakness and bulbar onset. Later, weight loss, reduced FVC, and limb muscle weakness are significant predictors of LMN dysfunction, and the transition away from the diagnosis of PLS. In particular, an MRC score of 4 or less in any muscle is associated with the eventual diagnosis of ALS, and its worse prognosis, but not PLS. We suggest that patients with only upper motor neuron signs have periodic EMG, muscle strength testing, weight measurement and FVC testing, because a change in any can signal the development of LMN signs and eventual ALS. Patients for whom these findings are normal after 4 years from symptom onset can be reassured by the diagnosis of clinically pure PLS, and may form a homogeneous group for enrollment in clinical trials.

C12 PROGRESSIVE MUSCULAR ATROPHY (PMA) IS SLOW AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Keywords: PMA, ALS, survival

Background: PMA is clinically characterized by pure lower motor neuron disease. It remains uncertain if PMA is part of or distinct from ALS. Only the absence of clinical upper motor neuron (UMN) signs distinguishes PMA from ALS. While PMA is thought to have a protracted disease course (1), a recent study emphasizes progression in the disease course (2).

Objectives: To investigate the proportion of patients with PMA who develop UMN signs in the disease course and to identify the outcome and clinical management in patients with PMA to find out how different they are from those of patients with ALS.

Methods: We reviewed the medical records of patients diagnosed with motor neuron disease (MND) between 2000 and 2007. We identified 91 patients diagnosed with PMA and 871 patients with ALS using follow up data. We excluded patients with flail arm/leg syndrome, primary lateral sclerosis, and other unclassified MND or cases with insufficient data. Kaplan-Meier survival curve for time from onset of weakness to death was estimated and the difference in survival curve between groups was examined by log rank test. Cox proportional hazards model was used to assess the effect of risk factors on the survival time.

Results: PMA had a higher proportion of males than ALS (73.6% vs. 54.9%, $p = 0.0006$) and an older age of onset than ALS (mean of 63.4 years vs. 59.9 years; $p = 0.0067$). PMA patients seemed to live longer with median survival time of 48.3 months vs. 36 months for ALS patients (log rank test, $p = 0.01$). The Cox model suggested that the hazard of death increased with age of onset in both groups ($p < 0.005$). Furthermore, ALS patients were at a higher risk of death than PMA patients after controlling gender and the age of onset ($HR = 1.74$, $p < 0.0001$). Twenty (22%) of these PMA patients developed UMN signs during the follow-up period (up to 68.7 months). Between PMA with and without UMN signs, no differences were found in gender, body regions involved at the onset and diagnosis, forced vital capacity, ALSRFS-R at diagnosis and changes of those during the follow-up. No convincing differences were found between the two groups in survival time. The use of percutaneous endoscopic gastrostomy (PEG) was 10% vs. 15%, while noninvasive ventilation (NIV) was used in 30% vs. 60%. Among patients with ALS, 14.5% used PEG, and 29.8% used NIV (the data after 2003).

Conclusion: PMA, when diagnosed, regardless of subsequent development of UMN signs, had a longer survival than ALS. PMA was progressive, care and management required in these patients did not differ from patients with ALS. Considering recently accumulated knowledge in pathology, neuroimaging, and experience in familial SOD mutation cases, PMA appears to be slowly progressive ALS, rather than a distinct entity (The study was supported by MDA Wings Over Wall Street.)

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C13 DIFFERENTIATION OF HEREDITARY SPASTIC PARAPARESIS FROM PRIMARY LATERAL SCLEROSIS IN SPORADIC ADULT-ONSET UPPER MOTOR NEURON SYNDROMES

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Keywords: primary lateral sclerosis, hereditary spastic paraparesis, upper motor neuron

Background: Differentiation between sporadic presentations of hereditary spastic paraparesis (HSP) and primary lateral sclerosis (PLS) is important for genetic counseling and for determining prognosis, as HSP generally has a more favorable prognosis than PLS and progression to ALS, as may occur in PLS, is not to be expected in HSP. To separate HSP from PLS previous studies used clinical features, such as age at onset <40 years, evidence of mild dorsal column impairment, and symptoms of urinary urgency, but there is little evidence to support this.

Objectives: To study whether clinical characteristics can be used to differentiate sporadic presentations of hereditary spastic paraparesis (HSP) from primary lateral sclerosis (PLS) in patients with apparently sporadic adult-onset upper motor neuron (UMN) syndromes.

Methods: Patients with a gradually progressive, adult-onset, UMN syndrome of at least three years duration were included. Exclusion criteria were a positive family history and other causes of UMN loss. Mutation screening of the spastin gene (SPG4), the paraplegin gene (SPG7) and the seipin/BSCL2 gene (SPG17) was performed in all patients.

Results: Included were 52 patients with a phenotype similar to typical HSP (involvement of the legs only), 36 patients with a phenotype suggestive of PLS (bulbar region involvement) and 16 patients with a phenotype of UMN involvement of arms and legs that was difficult to classify as a specific phenotype. Causative mutations were identified in 14 patients (7 SPG4 and 7 SPG7; 13 with leg involvement only and one with symptoms in arms and legs). Although age at onset was lower in the patients with confirmed HSP (39 years, range 29–69), there was considerable overlap with the subgroup of patients with bulbar involvement (52 years, range 32–76). No differences were found for evidence of mild dorsal column impairment (decreased vibratory sense, or abnormal leg SSEP), symptoms of urinary urgency or mild EMG abnormalities. Disease onset in arms or bulbar region, development of bulbar region involvement or marked asymmetry during the disease course may support a diagnosis of PLS.

Conclusions: In patients with an apparently sporadic adult-onset UMN syndrome and symptom onset in the legs, differentiation of sporadic presentations of HSP from PLS based on clinical characteristics is unreliable and therefore depends on genetic testing, although development of bulbar region involvement or marked asymmetry during the disease course may support a diagnosis of PLS.

C14 DEFINING SURVIVAL AS AN OUTCOME MEASURE IN ALS

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Keywords: endpoints, outcome measures, survival rate

Background: Which primary outcome measure best defines disease progression in ALS is debated. Functional endpoints are used, but consensus guidelines suggest that survival should be the primary endpoint for phase III trials and survival remains the standard for drug approval by regulatory agencies. Survival analyses have been used in different trials, but measuring the time to death in ALS is complex. Nutritional and respiratory interventions have become more sophisticated; survival rates could differ depending on the type of intervention used, both between and within trials. Further, the definition of survival has changed from trial to trial, with some studies including time to tracheostomy or prolonged non-invasive ventilation as part of the survival outcome. The objective of this study was to examine the impact of respiratory interventions on survival as an outcome measure and to define survival rate for trials in ALS.

Methods: We reviewed the data from three phase III clinical trials conducted between 1997 and 2005, and examined differences in time to death, tracheostomy, and permanent assisted ventilation (PAV) where data were available. Respiratory interventions and their relation to vital capacity (VC) were assessed with descriptive statistics, chi-square and Fisher's exact tests for categorical variables, and t-tests for continuous variables. Kaplan-Meier methods estimated differences in survival time between interventions. A power analysis generated sample size estimates for different endpoints.

Results: Approximately 82% of patients died of respiratory failure; 18% of patients died from other causes, usually sudden death. The use of respiratory interventions across centers ranged from 0–6.6% ($p=0.001$) of patients for tracheostomy, and 11.1–23.1% ($p=0.05$) of patients for non-invasive ventilation. Twenty-two percent of patients with tracheostomy had VC $\geq 50\%$ at the time of the procedure. In one trial, the mean survival time was 457.9 ± 3.1 days at 18 months as defined by a combined outcome and 467.2 ± 2.9 days with death alone as the end point ($p=0.017$). An estimated sample size to detect a 10% difference between groups was 488 patients for the combined outcome and 472 patients for death alone.

Conclusions: Tracheostomy and PAV are not equivalent to death in ALS. The use of respiratory interventions differs between centers leading to variability in combined outcome assessments. The time to endpoint can differ significantly depending on its definition, and combining outcomes does not reduce the estimated sample size of a trial. Death rate alone is the least variable and most easily identifiable measure of survival rate in ALS.

C15 HOW WELL CAN WE PREDICT ONE AND TWO YEAR SURVIVAL FOLLOWING DIAGNOSIS OF ALS?

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Keywords: Prognosis, predicting survival, outcome measures

Background: Previous studies have assessed correlations between survival and baseline ALSFRS (both in its original and revised format), baseline FVC and other factors such as age, sex, onset site and time from first symptom to diagnosis. These studies have focussed on dividing patients in good and poor prognosis groups rather than individual patient prognosis.

Objective: To assess the feasibility of using a large collection of data for several sources to predict survival in individual ALS patients.

Methods: We assembled a large pool of placebo patients from seven clinical trials and one clinical practice. We used a Cox proportional hazards (PH) model to select baseline characteristics to be used in predicting survival at 6, 12, 18 and 24 months following diagnosis or enrollment in a clinical trial. Characteristics under study included age, sex, site of first symptom, initial ALSFRS and FVC as a percentage of expected for normals as well as a measure of rate of disease progression based on time since first symptom and current ALSFRS. This rate is defined as 40 (or 48 for ALSFRSr) minus initial ALSFRS divided by time since first symptom measured in years.

Results: Using the combined data from 2356 patients and a Cox PH model, we found that age, initial FVC% and ALSFRS rate of progression were significant predictors of survival. Unfortunately, we also found that survival varied significantly from study to study even after adjustment for differing baseline patient characteristics. The estimated probability of surviving 12 months ranged from 63% to 92% for a 58 year old male, with initial FVC 86% and a 8.7 unit average drop per year in ALSFRS during the time from first symptom to enrollment. These estimates are based on data from 6 studies with minimum 12 month followup. At two years the range was 36% to 72% based on data from 4 studies.

Conclusions: Age, initial FVC% and initial ALSFRS expressed as a rate of progression are significant predictors of survival for patients with ALS. However, the proportion of patients expected to survive 12 months varied from study to study. The sources of this variability are not explained by the data gathered from these studies.

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SESSION 3A TRANSLATIONAL STRATEGIES

C16 HOW CAN ACADEMIC LABS CONTRIBUTE TO THERAPY DEVELOPMENT IN ALS? A MEDICINAL CHEMISTRY PERSPECTIVE

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Keywords: medicinal chemistry, rules and filters, chemistry garbage

An academic labs contribution to therapy development can be ruined by something totally outside of biology, namely miserable chemistry.

Here are some of the ugly facts about miserable chemistry. Seven out of eight commercially available screening compounds are chemistry trash; not worth screening for therapy development and quite likely not even worth screening as tools or probes in a chemical biology target validation exercise. It is not easy to discern true screening positives from false positives. The worst false positives are chemistry related. There is something in the chemical structure that causes the compound to appear active in a screen but in a biological sense the activity is bogus. This is really troublesome because chemistry related false positive activity holds up in replicate assays. The big pharmaceutical companies independently recognized this problem and junked a third to a half of their legacy screening collections. The bottom line; remove the chemistry garbage before you screen not after you screen.

Other dirty laundry facts you should know. Always confirm chemical structure. The label on the vial and the actual chemical structure can be very different. Always verify the chemistry on an active by re-synthesis of the compound before spending significant effort. Somewhere between 10–50% of actives do not hold up when the compound is remade and retested, even if the original sample identity and purity were OK.

There is a wealth of knowledge about miserable chemical structures but it is buried in the medicinal chemistry literature and not easily accessible to biologists. There are all kinds of rules and filters to get rid of the chemical garbage. Dealing with bad chemistry is not rocket science but it can be very difficult for a biologist. Think of a veteran medicinal chemist as a master of pattern recognition who can mentally connect a chemical structure to biological information. Seasoned medicinal chemists are like board certified pathologists in pattern recognition skills.

There is a big people-disconnect between academic biology and medicinal chemistry. The vast majority of medicinal chemists live in industry. Learning medicinal chemistry pattern recognition takes time, maybe 10–15 years to have a chance to really get good. The academic medicinal programs are few and because of the time factor the bulk of the knowledge lies in only a small number of senior faculty as opposed to graduate students or post docs.

A collaboration or connection to a medicinal chemist is a priceless asset if, as a biologist, you really want to make a contribution to ALS therapy development. You can expect a certain degree of conflict with the medicinal chemist. They may tell you the chemical compounds you are testing for your biology community peer reviewed publication are worthless. They will make "snap" judgements about chemical quality in just a few seconds by looking at chemical structures. My advice; if you want to positively connect to medicinal chemists put a beautiful looking chemical structure in the first few slides of your biology presentation.

C17 SMALL MOLECULE SCREENING FOR MOTOR NEURON PROTECTION: FROM BENCH TO CLINIC

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Keywords: TRO19622, clinical trial, drug screening

Rationalizing drug screening for ALS remains a challenge. The vast majority of cases are sporadic, and it is not known to what extent the disease pathophysiology in these patients reflects that in the familial forms (e.g. SOD1-related). In addition, different cell types (e.g. lower motor neurons, upper motor neurons, astrocytes and microglia) are involved and probably need to be targeted to obtain efficacy. Finally, and in common with many human neurodegenerative diseases, ALS affects post mitotic and highly differentiated cells that have specific properties that cannot be modeled by any experimental system. As a result, there is no perfectly validated molecular target and, to date, no animal model predictive of clinical efficacy in ALS. In such a context, the use of a phenotypic screening process to discover drugs that intervene in the fundamental pathways controlling survival and death of motor neurons appears a reasonable approach.

Starting in 2000, Trophos adopted that unconventional route and screened a library of 45,000 "drug-like" compounds on purified rat embryonic motor neurons deprived of trophic factors and selected TRO19622 (chemical name: cholest-4-en-3-one, oxime) as the most promising drug candidate (1). This cholesterol-like small molecule is orally bioavailable and crosses the blood brain barrier. It rescued motor neurons from facial nerve axotomy in neonatal rats and promoted axonal regeneration after peripheral nerve crush in mice. It also significantly delayed disease onset and improved the survival of SOD1G93A mice. Extensive systematic pharmacological profiling showed that TRO19622 interacts physically with two constituents of the mitochondrial permeability transition pore suggesting that its neuroprotective action is mediated, at least in part, through the modulation of the mitochondrial membrane permeabilization. Interestingly TRO19622 demonstrated efficacy in several models of pathological conditions where mitochondrial dysfunction has been evidenced such as painful peripheral neuropathies (2).

TRO19622 has completed regulatory preclinical assessment of pharmacology, safety, toxicity and pharmacokinetics showing no major signs of toxicity either in acute or long term regulatory safety toxicity studies. TRO19622 has successfully completed Phase 1/1b studies in both healthy volunteers and ALS patients demonstrating the product is well tolerated, has an excellent safety profile and that once-a-day dosing achieves the predicted exposure level required for efficacy, based on preclinical models. A pivotal European multi-centric Phase 2/3 trial of TRO19622 as add-on to riluzole is planned to begin in 2009 including 470 ALS patients. Although promoting motor neuron survival and nerve regeneration may not be sufficient to arrest all the events underlying this complex disease, the preclinical data obtained to date suggest such a compound will provide a step forward in the understanding of the disease process and will be of benefit to patients.

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C18 SMALL MOLECULE ACTIVATORS OF THE NRF2-ARE PATHWAY FOR TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: NRF2, drug, astrocyte

Background: Nrf2 drives expression of a battery of Phase II detoxification and anti-oxidant enzymes via its interaction with the antioxidant response element (ARE). When activated, this 'programmed cell life' (pro-cell life) response is neuroprotective and may be of clinical value in patients with Amyotrophic Lateral Sclerosis (ALS) in which oxidative stress is heavily implicated. There are a number of molecules which activate this pathway but they tend to be pro-oxidant in themselves and have little capacity for crossing the blood-brain barrier (BBB) and so may not be useful in *in vivo* models of human neurodegenerative disease.

Objectives: The primary aim of the present study was to conduct a search for small molecule activators of the Nrf2-ARE 'pro-cell life' pathway, with increased potency, minimal toxic effects and enhanced opportunity for CNS penetrance compared to the currently available tool molecules.

Methods: A small molecule library of 2,000 compounds (the Spectrum collection), containing natural products as well as 640 FDA approved drugs and molecules likely to modulate CNS function, was screened in an NRF2-ARE reporter assay designed and optimised for high-throughput screening. Hit molecules were assessed in *in vitro* models of oxidative stress and CNS specific reporter assays and profiled using chemi-informatics to select drug-like molecules with the potential for CNS penetrance. A small number of molecules were selected for further *in vivo* studies.

Results: In general, the hit molecules identified were able to robustly activate the NRF2-ARE pathway in an astrocytic cell line (C6), but less well in motor neuronal-like cells (NSC34). In addition C6 cells pre-treated with the NRF2 inducer which was then removed were significantly protected against an oxidative insult.

Two molecules with a history of human use were identified which satisfied our stringent criteria for activity in models of oxidative stress and potential for *in vivo* use and CNS penetrance. The first (Compound 1) is a natural product used in herbal medicine which had sub-micromolar activity in cellular reporter assays ($EC_{50} = 0.36\mu M$ in a C6 NRF2-ARE reporter cell line). The second molecule (Compound 2) has been used in human patients with another neurodegenerative condition. Interestingly the S(+) enantiomer of this compound which has lost primary agonist activity, retained NRF2-ARE inducing activity ($EC_{50} = 7.43\ \mu M$ in a C6 NRF2-ARE reporter cell line).

Discussion and Conclusions: These molecules show promise for further development as neuroprotective agents for ALS. Compound 2 may be particularly useful as it has a history of safe use in man, it is known to preferentially partition to the CNS and its use would not be limited by primary agonist activity, allowing higher doses to be investigated.

C19 PROTEIN BIOMARKERS FOR ALS DISEASE PROGRESSION

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Background: Amyotrophic lateral sclerosis (ALS) is a rapidly progressive disease, with the typical time from diagnosis till death of 2–5 years. There are no current diagnostic tests for ALS or surrogate markers of disease progression. Surrogate markers of ALS disease progression would be valuable measurements for drug efficacy in clinical trials and also to identify new targets for drug therapy. ELISA and mass spectrometry were used to identify protein alterations that correlate to disease progression. We identified specific proteins that correlate to clinical parameters of disease progression within ALS patients, and also proteins that identified subpopulations of ALS patients based on site of disease onset.

Objectives: To identify protein biomarkers that correlate to clinical measurements of ALS disease progression, we prospectively collected cerebrospinal fluid (CSF) and blood plasma every 4 months from 24 ALS patients. We also collected CSF and plasma from 14 control subjects over a 2-year time frame.

Methods: We collected blood plasma and CSF samples from 24 ALS (20 SALS and 4 FALS) and 14 control (11 healthy controls, 2 MS, 1 spinal cerebellar ataxia) subjects at the University of Pittsburgh School of Medicine. Samples were collected from ALS patients every 4–6 months over a 3-year time course. Samples were collected from control subjects over a 2-year period. Subjects were age/gender matched. ALS disease progression was monitored with clinical parameters including ALS-FRS, forced vital capacity (FVC), and manual muscle strength. We performed ELISA for cystatin C, transthyretin, neurofilament H, and anti-thrombin III on each CSF and plasma sample. Samples were run in duplicate and experiments repeated three times. Protein levels were correlated to clinical parameters of disease progression.

Results: Cystatin C protein levels in both the CSF and plasma decrease over time in ALS patients that exhibit rapid clinical disease progression. Transthyretin protein levels did not change over time in ALS patients, though there were modest increased levels in the plasma of limb onset ALS and decreased levels in the plasma of bulbar onset ALS. We observed increased levels of phospho-neurofilament H in the CSF of ALS patients and increased levels of anti-thrombin III in both the CSF and plasma of ALS patients during disease progression. Both neurofilament H and anti-thrombin III protein levels were increased in ALS patients when compared to control subjects.

Conclusions: Our data indicates that cystatin C may have utility as a surrogate biomarker for ALS disease progression, and could differentiate fast versus slow progression. In addition, phosphor-neurofilament H and anti-thrombin III protein levels were also altered during ALS disease progression, suggesting additional biochemical pathways altered during the course of disease. Overall levels of transthyretin did not significantly differ during ALS disease progression, though we noted differences in transthyretin levels between limb versus bulbar disease onset.

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SESSION 4A TDP-43

C25 PREFERENTIAL SEQUESTRATION OF TDP-43 AND LOW MOLECULAR WEIGHT NEUROFILAMENT (NFL) MRNA TO STRESS AND DEGRADATIVE GRANULES IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Keywords: TDP-43, neurofilament, mRNA stability

Background: It has been previously shown that the steady state level of the low molecular weight neurofilament (NFL) mRNA is selectively suppressed relative to that of the remaining middle (NFM) and high (NFH) neurofilament mRNA levels in degenerating motor neurons in ALS. Because altering the stoichiometry of NFL:NFM:NFH expression is associated with neurofilament (NF) aggregate formation in transgenic mice, this has been hypothesized to contribute to the formation of pathological NF aggregates in ALS. We have shown that NFL mRNA is destabilized in ALS, and that this is due to an alteration in mRNA binding protein expression. We have previously demonstrated that the TAR DNA binding protein (TDP-43) is a NFL mRNA stability determinant and that, in contrast to healthy motor neurons in which TDP-43 is almost exclusively nuclear in its localization, wheras in ALS affected motor neurons TDP-43 is strikingly over-expressed and almost exclusively cytosolic.

Objectives: Knowing that TDP-43 is a prominent component of RNA granules, including transport, stress and degradative granules, we have examined the relative distribution of NFL mRNA and TDP-43 amongst them.

Methods: We have examined both neurologically intact (control, n = 3) and ALS lumbar spinal motor neurons (sporadic ALS, n = 3) using confocal microscopy with TDP-43 (mouse monoclonal antibody), Staufen (specific to RNA transport granules), TIA-1 (specific to stress granules) and XRN-1 (specific to RNA degradative granules). Hoescht staining was used as a nuclear staining and motor neurons identified on the basis of morphological characteristics. To determine if NFL mRNA was associated with specific RNA granules, we used immunoprecipitated (IP) staufen, TIA-1 and XRN-1, followed by RT-PCR (IP-RT-PCR) for NFL.

Results: In normal motor neurons, TDP-43 colocalized strongly with Staufen and only weakly with TIA-1 and XRN-1. In sharp contrast, in ALS affected motor neurons there was strong colocalization of TDP-43 with XRN-1, and to a lesser extent with TIA-1, suggesting that TDP-43 is being sequestered from transport granules into translationally quiescent granules. NFL mRNA was detected in all IPs except with XRN-1 in normal motor neurons. There was a statistical increase in NFL mRNA in IP-RT-PCR with TIA-1 and XRN-1 in ALS lysates.

Conclusion: These data strongly suggest that TDP-43 relocalization in ALS affects the trafficking of NFL mRNA into translationally quiescent granules, and may thus contribute to the changes in NF stoichiometry seen in ALS affected neurons. *Research supported by the M. Halls Endowment.*

C26 DISTRIBUTION OF TDP-43 AND UBIQUITINATED INTRACYTOPLASMIC INCLUSIONS IN THE NEUROPATHOLOGIC GRADIENT OF NEURODEGENERATION IN SALS

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Keywords: TDP-43, motor neuron pathology, immunohistochemistry

Background: Motor neuron degeneration in SALS is a focal process that propagates through the neuraxis and leaves a gradient of neuron loss related to site of onset. This neuropatholgic gradient at least theoretically should represent the time-course of neurogeneration—the regions in close proximity to the site of onset in advanced stages and the more remote regions in relatively earlier stages. The distribution in this neuropathological gradient of abnormalities detected by immuno-histochemistry (IHC) with TDP-43 and ubiquitin and the correlation between the 2 antibodies are uncertain.

Objectives: i) To examine the distribution of abnormalities detected by TDP-43 and ubiquitin IHC relative to site of onset and degree of neuron loss; ii) to compare TDP-43 and ubiquitin IHC.

Methods: We had 5 groups—bulbar, arm, trunk, and leg onset disease and control. We looked at 4 neuraxis levels in each—medulla (hypoglossal nucleus), cervical cord, thoracic cord, and lumbar cord. We had 3 nervous systems in each group. We used TDP-43 and ubiquitin IHC on contiguous FFPE tissue sections. We evaluated the distribution of IHC abnormalities including the 2 morphologies (skeins and round inclusions) relative to the degree of neuron loss and compared between antibodies (TDP-43 and ubiquitin) on contiguous sections.

Results: IHC abnormalities were seen in all SALS nervous systems. IHC abnormalities was greatest in the regions remote from the onset, those with the most neurons, but this is explained by the greater number of neurons in these regions, not the frequency with which abnormalities appeared in each neuron. The abundance of IHC abnormalities between the different nervous systems within each group varied from scarce to moderate and was a variable different and independent of neuron counts. Skeins and dense round inclusions do not have obvious relation to each other: for example, skein-formation does not obviously precede round inclusions. Abnormalities detected by TDP-43 and ubiquitin have high concordance.

Discussion and Conclusions: IHC abnormalities do not clearly reflect the stage or degree of degeneration. Skeins and dense round inclusions do not clearly reflect states of protein deposition at different stages of degeneration. Allowing for extra "noise" of ubiquitin IHC, it appears that TDP-43 is the prime ubiquitinated protein depositing in motor neuron cytoplasm.The overall findings *may imply* that the actual death process of a particular motor neuron may be rapid (without a long process of demise) and proceed sequentially in queues rather than contemporaneously at each level as the overall degenerative process advances and summates.These findings are being quantified and are subject to change.

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C27 DISTINCT PATTERNS OF TDP-43 AND PROGRANULIN EXPRESSION FOLLOWING NEURONAL INJURY

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Keywords: progranulin, TDP-43, RNA stability

Background: The nuclear factor TAR DNA-binding protein (TDP-43) has been identified as a component of ubiquitinated inclusions in degenerating spinal motor neurons in ALS. TDP-43 is unique in having dual DNA and RNA binding properties, has both nuclear export and import sequences, and has been described as a component of RNA granules. We have shown that TDP-43 regulates the stability of NFL mRNA and recently observed its inclusion in stress granules. This suggests that TDP-43 may actively participate in regulating the expression of key cytoskeletal proteins in response to neuronal injury. Understanding the normal function of TDP-43 and the effect of neuronal injury on its expression can therefore be expected to provide considerable information regarding its role in the pathogenesis of ALS.

Objectives: To determine the pattern of TDP-43 and progranulin (PGRN) expression in an acute model of neuronal injury and to compare this to the expression patterns that are widely reported for the chronic motor neuron degeneration associated with ALS.

Methods: We have performed proximal and distal sciatic axotomies in C57BL6 mice aged 6 weeks. We conducted neurobehavioural assessment and removed tissue on post-injury days 1, 3, 7, 14 and 28. We observed protein localization by immunohistochemistry, protein expression levels and cleavage states by Western blot, and mRNA expression levels by RT-PCR.

Results: Consistent with observations of TDP-43 immunoreactivity in healthy human motor neurons, TDP-43 was predominantly nuclear in motor neurons in ventral horns contralateral to the sciatic injury. Within 24 hours after injury, TDP-43 expression in ventral horns ipsilateral to axotomy was upregulated in both nuclear and cytosolic compartments, becoming maximal by day 7 and declining to contralateral levels by day 28. This pattern of increased TDP-43 protein expression, as well as increased mRNA expression, was demonstrated by Western blot and RT-PCR of lumbar spinal hemicords ipsilateral to axotomy. Of note, the converse response was observed for PGRN in which neuronal PGRN levels dramatically declined, returning to contralateral levels by day 28. Equally intriguing, the decline in neuronal PGRN expression was associated with a marked increase in microglial PGRN expression. Cytosolic TDP-43 colocalized with both staufen and TIA-1, markers for RNA transport and stress granules respectively. We did not observe colocalization of TDP-43 or PGRN with active caspase 3.

Discussion: These findings suggest that cytosolic TDP-43 may not itself be pathological, but rather a critical component of the response to neuronal injury. This is further supported by the observation that increased cytosolic TDP-43 expression and decreased neuronal PGRN expression are reversible phenomena in this acute model of neurodegeneration, suggesting that these changes are physiological responses to neuronal stress. We hypothesize that the role of TDP-43 in the cytosol is to bind, stabilize and aid in the transport of mRNA species important to the recovery of motor neurons from axotomy. Hence, the upregulation of TDP-43 expression with prominent cytosolic localization in ALS motor neurons, previously thought to be pathological, may in fact be an appropriate response to neuronal injury.

C28 TDP-43 PATHOLOGY IN ALS-LINKED MUTANT VAPB TRANSGENIC MICE

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Keywords: VAPB, TDP-43, transgenic

Background: The Pro56Ser (P56S) mutation in vesicle-associated membrane protein-associated protein B (VAPB) is a cause of some familial forms of amyotrophic lateral sclerosis (ALS). The ALS causing mutation is within the major sperm protein domain of VAPB but the mechanisms by which VABP56S induces disease are not properly understood. In order to gain insight into these mechanisms, we generated transgenic mice that overexpress wild-type VAPB (VAPBwt) or VAPBP56S.

Objectives: To investigate the mechanisms by which ALS mutant VAPBP56S induces motor neuron disease.

Methods: Transgenic mice were made that overexpress either myc-tagged VAPBwt or VAPBP56S under the control of the prion promoter, and were studied using immunohistological and biochemical methods.

Results: We generated five transgenic lines expressing VAPBwt and five lines expressing VAPBP56S. Two lines of each were selected for further study. Immunoblots revealed that both VAPBwt and VAPBP56S were expressed in spinal cord. Immunostaining with antibodies to VAPB and the myc-tag on transgene-derived VAPB revealed that both VAPBwt and VAPBP56S were present within the cytosol of motor neurons but that VAPBP56S formed aggregates. Immunostaining with antibodies to a range of ALS-associated antigens revealed the presence of ubiquitinated inclusions within the cytosol of spinal cord neurons in VAPBP56S but not VAPBwt mice. Confocal microscopy revealed that the ubiquitinated deposits co-localised with TAR DNA-binding protein 43 (TDP-43) but not VAPB. We also analysed ubiquitinated deposits in SOD1G93A transgenic mice which have been reported to not contain TDP-43 and confirmed this finding.

Discussion and Conclusions: We report ubiquitinated TDP-43 pathology in VAPBP56S transgenic mice. This is the first report of TDP-43 pathology in a transgenic model of neurodegenerative disease including ALS.

C29 UPDATE ON THE ROLE OF TDP-43 MUTATIONS AS A CAUSE OF ALS

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Keywords: TDP-43, mutation

Background: TDP-43 was recently identified as the major protein of ubiquitinated inclusions in ~95% of ALS cases. Following this breakthrough significant progress has been made in delineating the spectrum of "TDP-43 proteinopathies". However, post mortem observations do not prove a biological role for TDP-43 in neurodegeneration. Our recent identification of TDP-43 mutations in sporadic (SALS) and familial (FALS) cases strongly suggests this role (1).

Objectives: To determine the mechanisms by mutant TDP-43 might contribute to neurodegeneration.

Methods: Critical analysis of recent scientific communications encompassing TDP-43 genetic and functional biological investigations.

Results: In all, 14 mutations have so far been identified in SALS and FALS cases by various groups (1–5). These mostly cluster at the c-terminus, with one in RNA recognition motif 1 (RRM1). Several of these mutations lead to serine and threonine residue substitutions. Pathological studies of some of these cases have demonstrated TDP-43 immunoreactivity similar to that seen in SALS. This is in contrast to SOD1 FALS cases, who demonstrate a lack of TDP-43 immunoreactivity.

TDP-43 possesses both nuclear localizing (NLS) and export sequences (NES). Experimental mutation of the nuclear localizing NLS in a cell culture model resulted in mislocalisation and fragmentation of TDP-43 mimicking that seen in pathology although no toxic effect was seen (6). However, an NLS variant was found in a FALS case with frontotemporal dementia (ALS-FTD) and shown in vitro to result in aberrant subcellular localization (7). Toxicity of wild-type TDP-43 was demonstrated in yeast cells in which cytoplasmic aggregation of full length and select truncated versions of TDP-43 resulted in cell death (8). Additionally, RNAi mediated TDP-43 knockdown in mammalian cells was also demonstrated to result in loss of cell membrane integrity and apoptosis (9).

Discussion and Conclusions: The clustering of TDP-43 mutations clearly implicates the c-terminus of this RNA binding protein in ALS pathogenesis. These mutations may increase the likelihood of phosphorylation or protein cleavage, but do not appear to affect nuclear localisation. The c-terminus is known to interact with other proteins, notably hnRNPs, but the complete list of interacting partners and the precise nature of these interactions is yet to be elucidated. The RRM1 mutant may be predicted to alter RNA binding, but again, the full list of RNA-interactions of TDP-43 is far from complete. The NLS mutant is perhaps the most obvious pathological candidate although it has been found in three controls to date. The possible effects of all these mutations on TDP-43 functioning and ALS pathogenesis will be elaborated on in the light of emerging evidence.

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SESSION 4B COMMUNICATION

C30 ALS COMMUNICATION RESEARCH: FROM THE LABORATORY TO THE LIVING ROOM

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Keywords: communication, dysarthria, collaboration

People with ALS can experience a range of communication impairments, most typically, but not exclusively, an acquired progressive motor speech disorder (dysarthria) characterised by reduced respiratory, phonatory and articulatory functioning. A review of contemporary research relating to dysarthria and communication reveals a number of discrete fields of investigation, including:

Neuro-anatomical research: investigating the relationship between motor/sensory neurological functioning and related anatomical sites. *Physiological research*: investigating the movement of, and interaction between, different speech subsystems. *Perceptual and acoustic research*: investigating the physical properties of dysarthric speech and how they are heard. *Intelligibility/comprehensibility research*: investigating how listeners perceive dysarthric speech. *Assistive technology and augmentative and alternative communication research*: investigating how communication technology can be used by people with dysarthria. *Autobiographical/biographical research*: investigating how people with dysarthria experience life. *Participation research*: investigating the effects of dysarthria on how people engage with the social world. *Social interaction research*: investigating the consequences of dysarthria on everyday interaction.

Findings from this spectrum of research fields provide a wealth of knowledge, but researchers and clinicians can currently only assume links between findings from impairment based research and the consequences of ALS on everyday life. Thus, whilst our (laboratory) knowledge of normal and disordered speech is now extensive, gaps remain with reference to adaptations over time and the effects of reduced intelligibility on everyday (living room) communication and participation both for individuals living with ALS and the people with whom they interact.

As indicated by the range of research activities above, it will not be possible to fully understand or resolve the characteristics and impact of dysarthric related communication through one research programme alone. Rather the challenge must be for a meaningful understanding of ALS dysarthria and its relationship to body structure, activity and participation. Crucially, there is an emerging need for much closer collaboration between people with ALS, clinicians and researchers. Through closer working there is a much greater chance of developing more meaningful and relevant questions as well as, ultimately, meaningful answers.

C31 ADVANCES IN COMMUNICATION TECHNOLOGY

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C32 ALS ONLINE: THE INTERNET AS A TOOL FOR PATIENT EMPOWERMENT

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Keywords: Online communities, patient empowerment, patient education

In the information age, doctors are no longer the sole gatekeepers of medical knowledge. The internet offers people with ALS the chance to find out more about their condition, read the latest research findings, share their experiences with other patients, and communicate with healthcare professionals. Historical research suggests that there was an active online community in ALS since the early 1990's, with a Usenet group being set up in 1996. As internet access became more widely available, there was a rapid growth in the number of patients sharing their illness narratives online and sharing experiences of experimental treatments. This has not been without risks, however, and untold thousands of patients have paid for experimental treatments of dubious validity marketed to them over the web.

Over time there has been a degree of continuity and handing down of knowledge which has taught newly diagnosed patients to be more critical consumers of information provided online, even to the point that patients are now critiquing research studies or even designing their own. There has also been a shift from the "Web 1.0" concept where a reputable institution such as a hospital or non-profit provides static information for download, to the more recent "Web 2.0" movement where the majority of content is user-generated and moderated.

A key theme has been patient empowerment; the web allows lay patients to learn more about their disease and become more engaged in the process of their treatment. At the next level, patients are able to talk to one another to suggest, reassure, or warn other patients about the pros and cons of their decisions.

The future of the web in ALS is collaborative online research. Patients are already reacting to research studies, for instance by seeking to be prescribed lithium off-label in response to a recent Italian study, submitting their self-report ALSFRS-R data at PatientsLikeMe in numbers 10 times that of the original study. There may also be a role for telemedicine in continuing to monitor progression past the point that clinic

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SESSION 6A USE OF THE G93A SOD1 MOUSE IN THERAPEUTIC TESTING

C33 THE SOD1 MOUSE MODEL OF ALS?

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Keywords: G93A mice, SOD1, model

For the past 10 years or more, the SOD1 mouse model has been one of the most important research tools for scientists working in the field of ALS. However, in recent months the validity of this mouse model has been widely questioned. This concern stems primarily from the failure of several successful preclinical trials in the SOD1 mouse to translate into effective therapies for human ALS. The most recent example of this failure was the publication in *Lancet Neurology* of negative results from a large phase III randomised trial of minocycline in ALS patients (1). This trial was initiated by positive findings that show that minocycline delays disease progression in the SOD1 mouse and the results of the trial led the authors to conclude that "either the current approach to translational neuroscience is unsatisfactory or the transgenic mouse model is a poor representation of sporadic ALS". Thus, opinion now varies as to whether the SOD1 mouse is simply a model of motoneuron degeneration or specifically SOD1-ALS rather than sporadic ALS.

In January 2008, a paper from Sean Scott (2) and colleagues added to the growing unease about the SOD1 mouse. These authors presented data indicating that most of the preclinical trials that have been published to date in the SOD1 mouse are likely to be a measure of noise in the distribution of survival means in this model rather than actual drug effect. Thus, for example, genetic variation (gene copy number) will be a significant determinant of lifespan.

However, before we discard the SOD1 mouse as a good model for ALS, we must ask ourselves whether the "problem" with the SOD1 mouse lies more in how we have used this model than in the model itself? For example, it is clear that human and mouse trials are performed very differently. Perhaps one of the most significant differences is that most preclinical trials have been based on presymptomatic treatment of mice. Is it really surprising therefore, that such trails may never be predictive of the outcome of treatment of human ALS patients, who are clearly well beyond symptom onset when they are enrolled into clinical trials? In addition, it is clear that we also need to improve our understanding of mouse pharmacokinetics and how similar they are to humans, in order to determine appropriate doses for use in human trials.

Although we look forward to the development of new models of ALS, it is clear that it will take several years for us to become as familiar with the pros and cons of these new models as has been the case for the SOD1 mouse. With this in mind it is likely that SOD1 mice will continue to be used to screen new ALS drugs. The need is therefore to use the SOD1 model as effectively as possible, bearing in mind the variables that we now know are associated with both the model as well as the researchers that work with it! The process towards standardization has been started by a consortium of European ALS researchers with the publication of a set of guidelines for preclinical testing of potential therapeutic agents in the SOD1 mouse (3). These guidelines attempt to address the issues of genetic background, pre-and post-symptomatic treatment regimes, as well as the need for dose-response curves and pharmacokinetic studies.

The emphasis should now therefore lie with implementing these guidelines to ensure that we produce results that are robust and use the SOD1 mouse to its full potential, whilst bearing in mind its shortcomings. The responsibility to get this right lies with all of us involved in the ALS research community, not only the scientists, but the funding agencies and journal reviewers and editors.

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C34 DESIGN, POWER, AND INTERPRETATION OF STUDIES IN THE STANDARD MURINE MODEL OF ALS

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Keywords: G93A mice, pre-clinical, SOD1 FALS

Identification of SOD1 as the mutated protein in a significant subset of familial amyotrophic lateral sclerosis (FALS) cases has led to the generation of transgenic rodent models of autosomal dominant SOD1 FALS. Mice carrying 23 copies of the human SOD1G93A transgene are considered the standard model for FALS and ALS therapeutic studies. To date, there have been at least 50 publications describing therapeutic agents that extend the lifespan of this mouse. However, no therapeutic agent besides riluzole has shown corresponding clinical efficacy. We used computer modeling and statistical analysis of 5429 SOD1G93A mice from our efficacy studies to quantify the impact of several critical confounding biological variables that must be appreciated and should be controlled for when designing and interpreting efficacy studies. Having identified the most critical of these biological variables, we subsequently instituted parameters for optimal study design in the SOD1G93A mouse model. We retested several compounds reported in major animal studies (minocycline, creatine, celecoxib, sodium phenylbutyrate, ceftriaxone, WHI-P131, thalidomide, and riluzole) using this optimal study design and found no survival benefit in the SOD1G93A mouse for any compounds (including riluzole) administered by their previously reported routes and doses. The presence of these uncontrolled confounding variables in the screening system, and the failure of these several drugs to demonstrate efficacy in adequately designed and powered repeat studies, leads us to conclude that the majority of published effects are

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most likely measurements of noise in the distribution of survival means as opposed to actual drug effect. We recommend a minimum study design for this mouse model to best address and manage this inherent noise and to facilitate more significant and reproducible results among all laboratories employing the SOD1G93A mouse.

C35 REFINEMENT OF MOUSE MODELS OF MOTOR NEURONE DISEASE AND GENERATION OF NOVEL READOUTS FOR THERAPEUTIC ASSESSMENT

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Keywords: methods, mouse, models

Background: Motor neurone disease (MND) is a devastating neurodegenerative disorder where loss of upper and lower motor neurones leads to gradual loss of motor function and death typically within three years. Mutations in SOD1 are responsible for a significant proportion of inherited MND cases. Mouse models exist which rely on the systemic transgenic expression of mutant human SOD1 (G93A SOD1). These mice show stereotypical loss of motor neurones leading to mild tremor and gait abnormalities obvious from about 75 days of age, progressing to significant paralysis, with humane intervention at about 140 days of age ('survival' time). Testing of new therapeutic approaches in this model is time-consuming and resource intensive and carries a significant burden of disease and distress for the mice, as studies are typically designed to measure extension in survival time.

Objectives: The primary aim was to describe the disease course in a pure genetic background, as opposed to the standard mixed genetic background and validate our standardised protocol for therapeutic testing. The second aim was to determine whether early behavioural or biochemical readouts could be identified and used as a rapid 'pharmacodynamic' readout for the efficacy of new therapeutic agents.

Methods: Multiple trials (6) were conducted in the C57Bl/6 G93A mouse model using standard methods of measuring disease progression (rotarod scoring, neurological state scoring, weight) over a period of two and a half years. At the outset we applied principles of good experimental design based on 'The Design of Animal Experiments'. In addition, systems for measuring mitochondrial function and oxidative stress *in situ* in spinal motor neurones were investigated.

Results: Analysis of this large data set lead to two main conclusions. Firstly, the disease course is remarkably consistent and significantly less variable in the pure genetic background. Power analysis indicated that the reduced variability would reduce n numbers by at least 60% when looking at effects on disease onset or survival time. Secondly, motor deficits are detectable very early and correlate with published patterns of synaptic remodelling at motor end plates. We identified early changes in motor function at about 40 days of age, long before classical onset of disease.

Discussion and Conclusions: This finding supports recent data indicating that the neuromuscular junction is lost first, followed by dying back of the axon itself. Our methods to quantify these early changes may enable early assessment of the neuroprotective potential of new therapeutic agents, increasing throughput and reducing the distress burden for mice on pharmacological studies.

C36 IDENTIFICATION OF THERAPEUTIC COCKTAILS FOR ALS BASED ON: COMBINATIONS OF FDA-APPROVED DRUGS, NUTRACEUTICALS AND METABOLIC PRECURSORS

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Keywords: symptomatic administration, G93A mice, drug cocktail

Background: Many existing therapeutic agents have shown significant survival effects in G93A mice when given at symptom onset – the more relevant approach for extrapolation to human ALS patients. These agents are only now reaching the stage of human clinical trials, and the outcome of these trials will provide much more compelling evidence for the predictive value (or lack thereof) of the G93A mouse in terms of therapeutics testing. However, even if the survival and motor function preservation benefits extrapolate directly to humans, no agent to date has shown the promise of totally arresting disease progression.

Objectives: To systematically test combinations of FDA-approved drugs and nutraceuticals that each extend survival individually when given at onset, to identify a drug "cocktail" which dramatically slows or halts disease progression. The use of approved drugs, and agents derived from foodstuffs (generally regarded as safe), will allow any such combination to be immediately taken to the clinic for small scale human trials. Candidate ALS patients include those who, for a variety of reasons, are unlikely to enroll in clinical trials involving experimental agents.

Methods: G93A SOD1 overexpressing mice, bred and maintained in-house, are tested in groups of 10, relative to vehicle controls. Simultaneous breeding of 50–55 non-transgenic females with littermate transgenic (Tg) males typically yields approximately 220 age-matched Tg mice. Treatment and control groups are randomized to give equal numbers of males/females per group, and treatment is begun at onset (~90 days of age). Established (published) criteria are used to determine the time to sacrifice, and the investigator making the determination is blinded to the treatment status. Survival results are expressed as a ratio of survival after onset, relative to vehicle controls from the same group of pooled littermates, tested simultaneously.

Results: Thus far, over 80 different agents and combinations (2–5 agents) have been tested. A number of new therapeutic agents and chemical classes have been identified, and at least three combinations have demonstrated additive effects far exceeding those seen with any individual agent. Most combinations to date have produced results comparable to individual agents, while some have shown sub-additive effects. Overall, the results have tended to validate the proof-of-concept, and have yielded one combination (four agents) of such magnitude to warrant consideration of a small human trial.

Discussion: Combination drug therapy has been a mainstay of cancer and HIV chemotherapy for many years, and is becoming more common for the treatment of dyslipidemias and microbial infections.Until more is known about the molecular pathogenesis of ALS, the best hope for an effective treatment in the short-term is via the use of combinations of agents which are already approved or regarded as safe based on years of human consumption without ill effects.

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SESSION 6B MANAGING ALS/MND IN CLINICAL PRACTICE

C37 AGGRESSIVE APPROACHES TO SYMPTOM MANAGEMENT IN PATIENTS WITH MOTOR NEURON DISEASE

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Keywords: therapies, symptom control, multidisciplinary care

The diversity and severity of symptoms arising from motor neuron disease (MND) is vast. Despite the large variety of symptoms encountered in patients with MND, there is a paucity of evidence-based recommendations for treatment.

An aggressive and proactive treatment for symptomatic control in MND frequently implicates empiric or anecdotal treatment trials of medications, therapies or devices. These may have an obvious benefit to the patient and be commonly utilized in selected clinics despite a lack of formal data from randomized, controlled trials. The disparity between the large number of available treatment options for control of disabling symptoms and the limited number of approved therapies has resulted in great variability between specialized treatment centers and the care received in more general neurology practice settings.

This presentation will highlight a variety of successful therapies which, although not formally approved for patients with MND, are commonly utilized or discussed in the context of aggressive, proactive multidisciplinary care. In addition, data from trials regarding symptom control in other neurodegenerative disease states will be reviewed.

Management of spasticity, sialorrhea, dyspnea, secretion management, cramping, muscle spasm and fatigue will be discussed. In addition, emerging technology for augmentative communication will be included. Available data and empiric observations for the use of intrathecal baclofen, botulinum toxin, modafinil, noninvasive ventilation during PEG placement, diaphragm pacing, in-exsufflation, standing frame and motorized pedlar will be included.

This overview presentation will emphasize many of the most problematic symptoms and the multidisciplinary approaches toward their effective resolution.

C38 RANDOMIZED PLACEBO-CONTROLLED CROSSOVER TRIAL WITH THC (DELTA 9-TETRAHYDROCANNABINOL) FOR THE TREATMENT OF CRAMPS IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Keywords: Randomized trial, Delta 9-tetrahydrocannabinol, cramps

Background: Many patients with ALS experience cramps. Severity varies from mild, without affecting daily activities and sleep, to disabling, where almost any voluntary muscle activity induces long standing, severely painful cramping. So far, there has not been any proven benefit of medications used for the treatment of cramps in ALS.

Objective: To determine the tolerability, safety and efficacy of THC in the treatment of cramps in ALS. It is hypothesized that the severity of cramps will lessen significantly in patients treated with THC compared to treatment with placebo.

Methods: Standard 2×2 crossover study design was used. Patients who had a cramp severity score of 5 or more on the visual analogue scale (VAS) became eligible. After a 14-day run-in period patients were treated for 2 weeks with either THC 5 mg bid or matching placebo in a double-blinded manner. After a 14-day washout period, a new 2-week treatment period followed. The primary variable of effectiveness was severity of cramps measured daily by a VAS. Secondary outcome measures were number of cramp days and nights and severity of fasciculations. Possible side effects were also monitored.

Results: A total of 27 patients were enrolled. Two patients experienced spontaneous lessening of cramps during the run-in period and withdrew from the study, one patient died during the study. Tolerability of the chosen dosage (THC 5 mg bid) was excellent. Only one patient experienced side effects (dizziness). No patient showed a significant change of monitored haematology and blood chemistry values. Three quarters of the patients were able to identify a treatment period with lessening of cramps. Currently statistical analysis is under way. A repeated measure ANOVA is applied with treatment and carry-over as within factors. The severity of cramps at each day will enter as a covariate in the model.

Discussion: For the treatment of cramps in ALS a dosage of THC 5 mg bid is well tolerated and safe. The fact that three quarters of the patients identified a treatment period with lessening of cramps suggests that THC is also effective. This was not caused by central side effects (e.g. euphoria). Statistical results and efficacy data will be included in the presentation.

C39 EVALUATION OF A "FAST TRACK" PROCESS FOR THE EVALUATION AND INVESTIGATION OF PEOPLE WITH SUSPECTED ALS

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Keywords: Fast track, diagnosis, investigation

Background: There is a perception in UK neurological practice that the evaluation of people with suspected ALS can sometimes take a relatively long period of time and that current structures are inconsistent with the expedited processes that often pertain for the investigation of people suspected of having other critical illnesses such as cancer. We have been able to implement a "fast track" (FT) process for the investigation of people suspected of having ALS.

Aim: To compare timelines in the process of the evaluation of people suspected of having ALS going through the FT process with those traversing more traditional pathways.

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Methods: Times from referral to first neurological consultation and from referral to diagnosis, along with other details relating to management pathways were determined in 144 patients with ALS. Comparisons relating to the patient journey were made between those patients going through the FT process and those following non-fast track (NFT) pathways.

Results: Median time from referral to diagnosis was 51 days for the FT patients and 104.5 days for the NFT patients. The theoretical target for this timeline for the FT patients was 8 weeks. 60% of the FT patients achieved this target compared with 30% of the NFT patients (OR 0.29, 95%CI 0.08–0.99). At 9 weeks the figures were 76% & 30% (OR 0.14, 95%CI 0.04–0.51), 12 weeks 88% & 30% (OR 0.06, 95%CI 0.01–0.29) and at 18 weeks 88% & 60% (OR 0.21, 95%CI 0.05–0.96) for FT and NFT patients respectively.

Discussion: Twice the proportion of patients in the FT process achieved diagnosis within the theoretical target of the FT clinic as those seen in the general clinics. This difference increases sharply in favour of the FT process as time following referral increases until 18 weeks when only 1.47 times the proportion of FT patients to NFT patients achieved diagnosis. The FT process therefore offers the chance of a much-accelerated patient pathway to diagnosis up to 18 weeks after referral. An important barrier to initiating the FT process is the identification of ALS as a potential diagnosis. It is possible that however efficient the diagnostic process from a theoretical perspective, a small proportion of service users will always have a relatively prolonged diagnostic journey. This can arise through atypical presentation, confusing co-morbidities etc. The UK NHS seeks to achieve diagnosis and treatment within 18 weeks of referral for all NHS patients and this data shows that in most cases this is being achieved. This work is currently being used in discussions with the UK Department of Health in the development of a generally applicable 18-week pathway for people suspected of having ALS.

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SESSION 7A ROLE OF NON-NEURONAL CELLS

C40 GLIAL CELLS AND NEURONAL REPAIR: LESSONS FROM SPINAL CORD INJURY

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Keywords: glial cells, transplant, spinal cord

We have transplanted cultured adult olfactory ensheathing cells into lesions of intraspinal long tracts and spinal root avulsions in adult rats. The grafted cells encourage the growth of the cut nerve fibres, and suppress the excessive neuromatous branching found in untreated lesions. The grafted cells take up an elongated shape, and form a tightly aligned bridge between the ends of the cut fibre tract. The regenerating nerve fibres enter the graft and follow this new, aligned bridge pathway. Within the bridge the nerve fibres are intimately ensheathed by the Schwann-like cells, and enclosed in an outer, perineurial-like sheath of fibroblasts. In the case of the spinal tracts, once they reach the end of the graft they re-enter the host spinal cord, and continue along the distal part of the corticospinal tract to form terminal arborisations. The effect is to put a patch over the lesion, restoring the integrity of the original pathway, and results in the functional recovery of some specific functional tasks. In the case of the dorsal roots the fibres re-enter the spinal cord, arborise in the dorsal horn, and ascend in the dorsal columns. In the case of the ventral roots there is a 4–5 fold increase in the numbers of fibres entering the proximal part of the root.

C41 NON-NEURONAL NEUROPROTECTION IN ALS USING GLIAL RESTRICTED PRECURSOR TRANSPLANTATION: A NOVEL APPROACH FOR RESPIRATORY NEUROPROTECTION

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Keywords: non-neuronal cells, astrocytes, stem cells

Background: Studies in ALS models have suggested that cellular abnormalities are not limited to motor neurons and that non-neuronal cells play a role in disease progression. However, previous ALS stem cell transplantation studies have primarily focused mostly on motor neuron replacement. Since most ALS patients die from respiratory muscle paralysis, targeting the phrenic motor neuron pools for neuroprotection may be a strategy with clinical relevance in ALS trials. We have employed glial restricted precursors (GRPs) from both rodents and human tissues for transplantation into the cervical spinal cord. Given these observations and other findings of astrocyte dysfunction in ALS, this proposal aims to target the replacement of dysfunctional astrocytes using GRPs for possible therapeutic benefits.

Objectives: 1) Determine the fate and survival of rodent and human glial restricted precursors (GRPs) following transplantation into the $SOD1^{G93A}$ rat model of ALS. 2) Determine the capacity for motor neuron protection and assessment of the important physiological properties of glial precursors following transplantation. 3) Determine the ability of GRPs to preserve forelimb strength, diaphragmatic function, and survival following transplantation into the $SOD1^{G93A}$ rat.

Methods: GRPs from both rodent lines and human fetal tissue were transplanted into the ventral horn of the cervical spinal cords of presymptomatic $SOD1^{G93A}$ rats at 3 levels (C4, C5, C6).

Results: The transplantation of GRPs results in differentiation into mature astrocytes which reside in the ventral gray matter adjacent to motor neuron soma and processes. $SOD1^{G93A}$ rats transplanted with GRPs demonstrate a preservation of diaphragm function, a slowing of forelimb grip strength decline and a delay in the onset of forelimb weakness. The effect was focal with no change in hindlimb grip strength. Survival was prolonged in glial precursor-transplanted $SOD1^{G93A}$ rats from the maintenance of respiratory function. The effects also appear related, at least in part, to the maintenance of glutamate transporter function—an astrocyte-specific property.

Discussion and Conclusions: The transplantation of GRPs results in the focal maintenance of respiratory physiology and function, a focal maintenance of forelimb strength and a slowing of the course of disease progression in the $SOD1^{G93A}$ rat. This approach appears to be related to astrocyte-specific properties of transplanted cells and not a non-specific cellular effect. These data suggest that glutamatergic pathways may play at least a part in this neuroprotection. The use of human GRPs may offer a novel approach for stem cell replacement strategies in ALS patients with a focus on the preservation of respiratory function.

C42 FOCAL DEGENERATION OF GLUTAMATE-VULNERABLE ASTROCYTES IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: nonneuronal cells, degeneration, glutamate

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder characterized by the loss of corticospinal and spinal motor neurons. The causes of the disease are mainly unknown, but about 2% of human cases have been associated with mutations in the gene encoding the antioxidant enzyme copper, zinc superoxide dismutase (SOD1). While the cascade of events ultimately responsible for motor neuron degeneration remains elusive, recent observations suggest that the death of motor cells implies a combination of cell-autonomous and non-cell-autonomous mechanisms that also involve glial cells, particularly microglia and astrocytes.

To study the morphological and structural changes astrocytes undergo during the progression of the disease, we performed immunohistochemical analysis of spinal cord sections from transgenic mice expressing the ALS-linked mutant SOD1 Gly93→Ala ($hSOD1^{G93A}$). In the neighbourhood of motor cells, we found that a subset of astrocytes harbouring protein

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inclusions underwent morphological and biochemical changes that were reminiscent of degenerating cells. These alterations show before the loss of motor neurons and the phenomenon becomes significant concomitant with the onset of neuronal degeneration and the appearance of ALS symptoms.

To investigate the impact of mutant SOD1s (mtSOD1s) on astroglial properties, we then expressed either the human wild-type (WT) or the two mtSOD1s, G93A and G85R, in primary cultures of rat spinal cord astrocytes. We found that mtSOD1s themselves induce no deleterious effects on cultured astrocytes but make them rather susceptible to the pro-apoptotic action of non-toxic concentrations of the excitatory neurotransmitter glutamate. Such effects are mediated by the metabotropic glutamate receptor 5 (mGluR5), as demonstrated by the fact that mGluR5 blockage is protective against the gliotoxic insult. Moreover, *in vivo* administration of an mGluR5 antagonist reduces astrocyte degeneration, delays the appearance of ALS symptoms and extends survival in $hSOD1^{G93A}$ transgenic mice.

All of these data indicate that spinal cord astrocytes are endangered by the expression of ALS-linked mtSOD1s, become highly vulnerable to mildly toxic stimuli present in their microenvironment, and start to degenerate. This in turn may accelerate degeneration of the neighboring motor cells in an interactive process of reciprocal damage.

C43 MOTOR NEURON ROS MAY CONTRIBUTE TO ASTROCYTE PATHOLOGY IN A MUTANT SOD1 RAT MODEL OF ALS

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Keywords: G93A, Ca^{2+} permeable AMPA channels, GLT-1

Background: Loss of astrocytic glutamate uptake in ALS may underlie excitotoxic motor neuron (MN) damage in the disease. However, the reason for the loss of astrocyte glutamate transporter function has been unknown. In past studies we have examined factors underlying a high susceptibility of MNs to excitotoxic injury, and found that MNs possess large numbers of Ca^{2+} permeable AMPA-type glutamate (Ca-AMPA) channels (1), and that MNs buffer cytosolic Ca^{2+} loads poorly such that much of the Ca^{2+} entering through these channels is taken up by mitochondria, with consequent disruption of mitochondrial function and strong reactive oxygen species (ROS) generation (2).

Objectives: The aim of this study was to test the hypothesis that ROS produced in MNs contributes to the dysfunction of astrocytic glutamate transporters.

Methods: *In vitro* studies employed mixed spinal cultures (containing neurons on a monolayer of astrocytes). *In vivo* studies used G93A SOD1 mutant rat models in which drug was infused intrathecally during the late presymptomatic period (from $\sim$ 67 to 97 days).

Results: In cultures, we found glutamate to cause far more ROS generation in MNs than in other spinal neurons. Furthermore, this ROS appears able to exit the MNs and induce oxidation and rapid disruption of glutamate transport in adjacent astrocytes (3). Subsequently, to begin to assess the contribution of Ca-AMPA channel activation *in vivo*, we have carried out 30 day intrathecal infusion of the Ca-AMPA channel blocker, naphthyl acetylspermine (NAS), in G93A mutant SOD1 rats (4). In wild type animals, immunoreactivity for the astrocytic glutamate transporter, GLT-1, was particularly strong around ventral horn MNs. However, a marked loss of ventral horn GLT-1 was observed, along with substantial MN damage, prior to onset of symptoms (90–100 d) in the G93A rats. Conversely, labeling with the oxidative marker, nitrotyrosine, was increased in the neuropil surrounding MNs in the transgenic animals. Compared to sham treated G93A animals, 30 day NAS infusions (starting at 67 ± 2 days of age) markedly diminished the loss of both MNs and of astrocytic GLT-1 labeling.

Discussion and Conclusions: Our culture studies provide precedent for the possibility that MN ROS can disrupt astrocytic glutamate transport. The finding that NAS, which can decrease glutamate triggered ROS generation in MNs, slows the loss of astrocytic glutamate transport in G93A rats, suggests that this mechanism may contribute to transporter loss *in vivo*. As astrocyte dysfunction can clearly damage MNs, we propose that reciprocal deleterious interactions between MNs and surrounding astrocytes underlie a final common pathway of disease progression in ALS (5).

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C44 RILUZOLE AND DEXAMETHASONE BUT NOT CEFTRIAXONE UPREGULATE GLUTAMATE TRANSPORT ACTIVITY AND GLUTAMATE TRANSPORTER EXPRESSION IN STRIATAL ASTROCYTES

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Keywords: astrocyte, transporter, neurodegeneration

Glutamate transporters expressed in astrocytes are critical for maintaining the extracellular concentration of glutamate below toxic levels in the central nervous system. In light of a number of prominent reports in this area, we selected a number of drugs which might potentiate the activity of glutamate transporters and therefore be beneficial as neuroprotective agents in Motor Neurone Disease.

The objective of the work was to determine the ability of a number of drugs to cause elevations of protein levels of the main glutamate transporter, GLT-1 and enhance ^{3}H-glutamate uptake activity in primary cultures of striatal astrocytes. In particular we wished to directly compare ceftriaxone, which has been reported to be a positive modulator of GLT-1 levels with other agents that have been proposed to upregulate GLT-1.

Primary striatal astrocyte cultures from E16 mouse embryos after 7 days *in vitro* were grown for 4 days in medium supplemented with G5, a defined cocktail of growth factors then treated for three days with dexamethasone (0.1 and 1

μM), riluzole (1–100 μM), zonisamide (1–1000 μM), citicholine (10–1000 μM), ceftriaxone (100 and 1000 μM), or vehicle as a control. The vehicle-treated cultures showed low GLT-1 levels resulting from growth factor withdrawal, and Western blot analysis showed that only dexamethasone (1 μM) and riluzole (100 μM) maintained or induced increases in GLT-1 protein, whereas zonisamide, citicoline and ceftriaxone treatment did not elevate the protein level. Dexamethasone and riluzole caused elevations in GLT-1 mediated ^{3}H-glutamate uptake, determined by estimating the amount of uptake that is sensitive to WAY-213613.

Our results show that there are some compounds which show promise as a positive modulators of GLT-1 levels, although ceftriaxone does not regulate GLT-1 under these assay conditions.

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SESSION 7B MULTIDISCIPLINARY CARE MANAGEMENT

C45 USE OF PROSPECTIVE REGISTERS IN DEFINING CLINICAL CHARACTERISTICS AND IMPROVING CARE PROVISION

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Keywords: Epidemiology, Italy, Registers

Amyotrophic lateral sclerosis (ALS) is a relatively rare disease with an annual incidence rate between 1.5 and 2.5/100,000 population. Over the past 10 years, the design of ALS epidemiological studies has evolved to focus on a prospective, population based methodology, employing the El Escorial criteria and multiple sources of data to ensure complete case ascertainment. Six European and North American studies based on this paradigm have been published and show remarkably consistent incidence figures among their respective Caucasian populations. Although population based studies have been useful in defining clinical characteristics and prognostic indicators in ALS, many epidemiological questions remain that cannot be resolved. The working hypotheses is that ALS, like other chronic diseases, is a complex genetic condition, and the relative contributions of individual environmental and genetic factors are likely to be relatively small. Larger studies are required to characterise risks and identify sub-populations that might be suitable for further analysis.

The Piemonte and Valle' d'Aosta ALS register (PARALS) is a prospective register collecting all cases of ALS incident in two regions of north-western Italy (total population, 4,332,842). This was established in 1995, based on the collaboration of all the neurological departments of the area. Patients are prospectively followed-up from diagnosis. In the 10-year period of observation, 1995 to 2004, a total of 1260 new cases of ALS were detected, 687 men and 573 women. The mean age at onset was 64.9 years (SD 11.2) (men 64.6 (SD 10.1); women, 65.4 (SD 10.7); $p =$ n.s.). The mean time delay form onset to diagnosis was 10.4 months (SD 10.1), with a significant decrease in the last 5-year period (1995–1999, 11.0 months; 2000–2004, 9.7 months; $p = 0.001$). The mean annual crude incidence rate in the period 1995–2004 was 2.90 (95% c.i., 2.72 to 3.09). Crude incidence rates were significantly higher in men. The incidence rates did not change when comparing the first 5 years (1995–1999, 2.87/100,000) and the second 5 years (2000–2004, 3.00/100,000) of the study. A total of 319 patients were alive at the prevalence day (December 31st, 2004), corresponding to a crude prevalence rate of 7.40 (6.6–8.3)/100,000 population. There was a slight but not significant increase of median survival time. There was significant increase of the number of patients who underwent non-invasive ventilation (NIV) (10.3% vs. 17.9%; $p = 0.0001$), whereas the number of patients who performed tracheotomy and PEG/PRG only slightly increased.

In conclusion, ALS incidence showed no relevant modifications during the 1995–2004 period in this population-based prospective register. The significant reduction of the mean time delay is likely to indicate an increased awareness of ALS among the neurologists and other physicians in the examined area. Despite the increased use of NIV, there was only a modest increase of median survival.

C46 DIAGNOSTIC PROCESS AND NEUROLOGICAL CARE IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS IN SPAIN

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Keywords: Diagnosis, neurological care, Spain

Background: The adequate health care of ALS patients depends on knowledge of the disease and the adequate resources of the health care system. Identification of care deficits caused by failures of the system or the professionals is the first step that is needed to resolve them.

Objectives: To determine the health care status of Spanish patients with ALS, specifically their diagnostic process and neurological assistance in order to identify needs and to propose improvements.

Methods: We created a 156 item questionnaire that included demography, clinical status and administered health care. Diagnostic process and neurological care accounted for one third of the questions. The questionnaire was hand delivered or mailed to up to 400 patients with ALS from the Spanish ALS Foundation nationwide. Response to date is 260. A univariant analysis with SPSS 10.0 for Windows was performed.

Results: The patient's median profile is male/female (ratio 1:1); 54 years old (range 29–79); married (81%); spouse as primary caregiver (70.8%); and from Madrid (41.5%). Diagnostic and neurological care habits and deficits were identified. Among those, median diagnostic delay time was reported as 12.6 months; of those, 4.7 months in specific testing. To establish diagnosis, two or three physicians were required by 61.9% of patients; four or more by 27% of patients. The way the diagnosis was given was perceived as with scarce information or just tactless by 35.4% of the patients. In 33.8% of the cases, diagnosis was only given to the family. Seventy three percent of patients requested a second opinion; of which just over ten percent were given at our Unit. Sixty-one and a half percent of patients were being primarily attended by non-neurologists. The mean period between visits was 3.8 months. Patients reported that only 42.6% of their doctors knew and were interested in their disease. 38.2% of patients received neurological assistance in a different territorial area that of their own. Both the diagnostic and detailed therapeutic practices of neurologists as reported by the patients will be presented.

Conclusions: There is a considerable delay in establishing the diagnosis, which delays the onset of therapies and most likely increases the patient' emotional distress. A significant percentage of patients received the diagnosis in an unsatisfactory and unhealthy way; were primarily cared for by non neurologists; sought neurological care out of their own sanitary area; or considered their doctors not knowledgeable or uninterested in the disease. Resolving the deficits identified requires the determined intervention of health care decision makers to both provide professional training and generate additional infrastructure resources.

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C47 LAPAROSCOPIC PLACEMENT OF GASTROSTOMY FEEDING TUBE (LSCG-TUBE): INITIAL EXPERIENCE

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Keywords: gastostomy, nutrition, laparoscopic

Background: Gastric-feeding access has been traditionally established by endoscopic or radiologic means. Laparoscopic techniques have been described to assist endoscopic placement of the standard PEG feeding tube.

The traditional open surgical gastrostomy tube incorporates a purse-string suture sealing the stomach about the tube as well as placement of multiple sutures anchoring the stomach to the abdominal wall in a circumferential manner theoretically eliminating the devastating complication of intra-abdominal sepsis resulting from leak and/or early dislodgement real to the less invasive methods. A laparoscopic approach mimicking the original open surgical procedure preserves these technical benefits and also affords the unique advantage of correcting a hiatal hernia if present. By inference, the latter may reduce risk of aspiration of gastric contents in neurologically impaired patients.

Objectives: To explore the feasibility of LSCG-tube placement in ALS patients using general anesthetic, including repair of hiatal hernia when present.

Methods: N = 17 patients with definite or probable ALS with Laboratory Support (El Escorial criteria) from our ALS Center were referred for LPS G-tube placement. 10/17 presented with bulbar disease. These patients were evaluated by one surgical consultant (author PRN). Initially, patients were refused endoscopically or radiologically placed tubes for pulmonary or anatomic reasons. All patients showed impaired swallow. Pulmonary function varied widely (VC median: 53% predicted). All patients required general anaesthesia. Tubes were not sewn to skin nor were removable skin sutures or staples employed. Post-procedural sedatives and narcotics were judiciously minimized. A surgical follow-up was performed at one month and every three months thereafter.

Results: All 17 underwent successful LPS G-tube placement without leakage, bleeding, or intolerance to use within 24 hours. Five patients also underwent concomitant hiatal or paraesophageal hernia repair. All patients tolerated bolus gastric feedings except one patient with recurrent paraesophageal hernia salvaged by radiologic conversion to gastrojejunal access.

Peri-operative complications include immediate reintubation (n = 1), delayed extubation (n = 1) and tube dislodgement (n = 1). Three out of seventeen patients died within 30 days of procedure. Four additional patients died within 6 months of procedure. There were no known gastric aspiration events.

Discussion and Conclusion: Initial results suggest that LSC G-tube placement is a safe procedure in ALS patients despite suboptimal pulmonary function. A general anaesthetic has the paradoxical advantage of airway control and permits liberal peri-procedural sedation and analgesia. With rehydration, management of oral-pharyngeal secretions must be anticipated. This technique has the advantage of direct visualization of a hiatal defect and repair if present.

C48 MEASURING DISEASE PROGRESSION IN ADVANCED ALS: THE ALSFRS-R EXTENSION (ALSFRS-EX)

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Keywords: ALSFRS-R, advanced disease, clinical trials

Background: The Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised (ALSFRS-R) was developed for use in clinical trials where death or ventilation was considered the endpoint of study. Since the publication of the original scales, a small but increasing number of patients decide to have invasive ventilation or survive for several years on continuous non-invasive ventilation. Although dependent on others for activities of daily living, many report satisfaction with their lives and a desire to continue tracking their progress beyond the zero-point on the ALSFRS-R.

Objectives: To expand the range of the ALSFRS-R to measure disease progression in those with advanced disease.

Methods: Following interviews with patients in advanced disease states (ALSFRS-R scores <25), we developed 10 new items that could potentially be added to the scale. In August 2007 we invited 1246 patients to complete the ALSFRS plus the 10 new questions in an on-line survey. 326 patients (26%) responded between 24 August 2007 and 15 September 2007. A 1-week retest was initiated after the first response. 271 of the baseline respondents were invited between 1- and 7-September 2007 to retake the survey. 169 patients (62%) responded to the retest between 7 and 21 days after the initial survey; 80% responded in 7 to 10 days. 218 (67%) of the baseline respondents completed a 3-month follow-up survey, which was conducted to explore the ability of the extended measure to detect change in comparison with the original ALSFRS-R.

Results: Respondents were broadly representative of members of an online community for people with ALS. Adding all 10 items increased the overall Cronbach's alpha of the ALSFRS-R from 0.881 to 0.932. Factor analysis revealed 8 of the 10 new items conformed to the existing factor structure of the original scale. Test-retest reliability was $r = 0.9$ or higher. Following the 3-month retest we repeated our analyses and decided upon four new items: ability to get around the home, ability to move fingers, ability to show emotional expressiveness in the face, and ventilator use. Each new item loads onto the existing factors of the scale: gross motor, fine motor, bulbar, and respiratory. The new items improve discrimination at the lower end of functioning in ALS patients.

Discussion: As ventilation and assistive technologies become a more widespread option, many patients will opt to live for several years as a "zero" on the existing scale. We have concisely extended the range of the ALSFRS-R to better monitor the progression of these patients with advanced disease. The ALSFRS-EX could be particularly useful in clinical trials and studies examining end-of-life decision making.

C49 AN INVESTIGATION OF EMOTIONAL STRESSES EXPERIENCED BY MULTIDISCIPLINARY ALS CLINIC STAFF

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Keywords: Stress, clinic

Background: ALS is a progressive disorder with no effective therapy that leads inexorably to death. Multidisciplinary ALS clinics may make 20–100 new diagnoses per year and experience a similar number of deaths per year. Thus, the clinical evaluation, giving the diagnosis, ongoing management, and death of the patient are emotionally taxing for clinic staff. Little is known how clinic members handle these stresses.

Objectives: We conducted a survey to better understand the types of stresses and techniques to manage them. We also queried methods of making and giving the diagnosis.

Methods: A set of questionnaires, for the neurologist (who makes the diagnosis) and the clinic manager (who continuously interacts with patients and families), was sent to 68 multidisciplinary ALS/MND clinics in the US. Included were questions on demographics; how the diagnosis is made and given; stressful factors in making and giving the diagnosis, with continued care, and at time of death; and strategies to handle stress.

Results: *Demographics:* the majority of neurologists were male and the majority of managers were female. The average number of new diagnoses was >50 per year. Fifty percent of neurologists and 95% of managers acknowledged being a "spiritual person". *Making the diagnosis*: Two-thirds relied on the El Escorial criteria, but most acknowledged diagnostic limitations. Most ordered a battery of laboratory tests even though the diagnosis of ALS was clear. All delivered the diagnosis in person. There was a range of stress in giving the diagnosis that tended to lessen with experience. *Continuing Care:* There was greater stress for managers, and experience tended to lessen stress more for neurologists. Two-thirds of clinics did not write prescriptions for off-label medications, and such requests were stressful. *Time of Death:* There was moderate stress for both neurologists and managers that did not lessen with experience. More managers than neurologists personally contacted family and attended funerals. *Overall Stress:* There was a tendency for reduced stress with experience. Techniques to manage stress included exercise, spirituality, alcohol, and support from staff (discussions), but all were judged to be of low efficacy. The majority were interested in a national effort to address stress, in the form of seminars, videos or books. *Overall Satisfaction*: Neurologists and managers were uniformly satisfied, felt they were doing a good job, and few considered leaving the field. Challenging operational issues were finances, personnel, and time.

Discussion and Conclusions: ALS clinic providers experience considerable stresses, but they are manageable and some may lessen with experience. There are different stress management techniques, but none are clearly successful. Despite these stresses overall satisfaction is high. A formal effort to recognize and manage stress is favoured. It might be helpful to orient new personnel to these stresses and management.

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SESSION 7C CLINICAL ELECTROPHYSIOLOGY & IMAGING

C50 CONCEPTS AND TECHNIQUES IN CLINICAL ELECTROPHYSIOLOGY: LOWER AND UPPER MOTOR NEURON MEASUREMENT IN ALS

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Keywords: ALS progression, lower motor neuron, upper motor neuron

Background: Lower (LMN) associated with upper motor neuron (UMN) dysfunction defines ALS. Amplitude of the motor response, neurophysiological index (NI) and motor unit number estimation (MUNE) are sensitive methods for detecting LMN loss over time. These measurements are more sensitive than functional scales or forced vital capacity, and the rate of motor unit loss as evaluated by MUNE is a prognostic factor. The main limitations of these measurements are the large standard deviation (SD) of the mean, change overtime and the need of some training to perform the neurophysiological studies, especially MUNE. The large SD has a negative impact on the calculated number need for treatment for testing an estimated effect in clinical trials. The inclusion of a homogenous population would circumvent this inconvenience. Regarding UMN evaluation the conventional transcranial magnetic stimulation (TMS) measurements are considered in sensitive. The new triple stimulation is accepted as a promising tool. Although there are a large number of transversal studies in ALS analyzing the utility of several TMS measurements to detect abnormalities related to UMN dysfunction, only a few studies focused on longitudinal evaluations to find TMS measurements that could show sensitivity to disclose changes. Probably, motor evoked potential amplitude is very variable to be reliable, but corticomotor threshold and central conduction time show progressive increase over time and can be useful. Moreover, cortical silent period has been reported as meaningful, and should be explored further in ALS.

An interesting point is that in spite of many studies evaluating UMN and LMN loss in ALS, none investigated the measurements for LMN and UMN lesion in the same population of patients. This strategy may allow the testing of the interdependency of LMN and UMN degeneration.

Objectives: We review the relevant literature concerning clinical neurophysiology to measure the progressive UMN and LMN dysfunction in ALS. In addition, we aimed to test the sensitivity to change of neurophysiological measurements for UMN and LMN evaluation, in a group of ALS patients suitable for entry into clinical trials.

Methods: We included 28 early affected ALS patients with probable or definite disease (revised El Escorial criteria): ALS-FRS >30, disease duration <24 months, forced vital capacity (FVC) $>70\%$. All of them had abductor digiti minimi (ADM) strength >3 on the MRC scale on both hands. We evaluated the following measurements at entry and 6 months later: ALS-FRS, FVC, amplitude of ADM motor response (CMAP); NI, MUNE, cortical silent period before and after ADM fatigue, the ratio motor evoked response by TMS/CMAP; TMS index (TMSi) as previously described-Z score of the cortical threshold + Z score of the central conduction time. A p value <0.01 was accepted as significant (Friedman and Wilcoxon signed rank tests).

Results: All patients were completely evaluated at both visits. The ratio MEP/CMAP and the increase of the CSP after fatigue did not change significantly. ALS-FRS (18.4%), FVC (15.4%), CMAP (25.5%), NI (41.9%), MUNE (40.4%) decreased significantly in 6 months. TMSi and CSP increased significantly (170.4 and 29.2%, respectively). NI, MUNE and CMAP had a lower coefficient of variability (0.57, 0.61 and 0.65 respectively). This coefficient was very large for TMSi. NI and MUNE changed more than ALS-FRS, FVC and CMAP, but similarly to TMSi and CSP. CMAP was more sensitive than FVC. TMSi and CSP increment was statistically comparable.

Discussion: In addition to confirming that NI and MUNE are sensitive markers of LMN loss, this study shows that conventional TMS can be used to evaluate UMN loss in ALS over time. Both TMSi and CSP increased significantly over 6 months, and the percentage of change was comparable to LMN loss. Although the amplitude of the motor response evoked by TMS is quite variable, cortical threshold and central conduction time increased in this period. In addition, the prolongation of the CSP suggests a progressive dysfunction of inhibitory pathways. Thus, that conventional TMS can be a useful to measure UMN dysfunction in ALS. Its role in ALS should be reconsidered.

C51 MOTOR UNIT NUMBER INDEX (MUNIX): A NOVEL NEUROPHYSIOLOGICAL TECHNIQUE TO FOLLOW DISEASE PROGRESSION IN ALS

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Keywords: MUNIX, motor unit number, electrophysiology

Background: Several motor unit number estimation (MUNE) techniques have been applied to quantify motoneuron loss in ALS. Disadvantages of the existing methods are that they are time consuming (20–30 min. per muscle) and/or invasive. MUNIX is based on surface-EMG recordings, requiring only a few minutes per muscle (1).

Objective: To evaluate the feasibility of MUNIX as a marker to objectively measure disease progression in ALS.

Methods: As of summer 2007 seven patients (5 males, 2 females, mean age 61, disease duration less than 14 months) were enrolled in a randomized controlled clinical trial (SIRONA) At 2-monthly follow-up visits clinical data, CMAPs and MUNIX from 8 muscles (APB, ADM, AH, EDB bilaterally) were obtained. After measuring one single supramaximal CMAP, surface EMG was recorded during voluntary contraction of muscles at increasing force levels (minimal to maximal, 9 times).

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Results: By the end of March data were available over a 6-month-period from each patient. The method was very well tolerated, no participant retired from the trial. Technically no major problems occurred. At study entry mean MUNIX per muscle was 56.5 ± 20.4, mean CMAP per muscle 4.3 ± 1.3 and mean ALSFRSR 43.6 ± 2.4. After 6 months MUNIX had dropped to 53.1 ± 18.0, CMAP to 3.9 ± 1.6 and ALSFRSR to 39.0 ± 5.2. Changes were only significant for the ALSFRSR ($p < 0.019$ paired t-test).

Discussion: MUNIX analysis is quick to perform and well tolerated, but patient co-operation is necessary. Changes over a 6-month-period were not significant. One-year data and their statistical analysis will show whether MUNIX is superior to standard neurophysiological measures (CMAP) and clinical scores.

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C52 FACILITATION OF THE JAW-JERK REFLEX IN BULBAR ONSET ALS PATIENTS

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Keywords: ALS, jaw-jerk reflex

Background: The jaw-jerk Reflex (JJR) is the trigeminal equivalent of monosynaptic myotatic reflexes in limb muscles. JJR excitability is determined by suprasegmental influences on brainstem motor circuits, and could be used as an indirect assessment of upper motor neuron tone. There are no previous studies of JJR in patients with amyotrophic lateral sclerosis (ALS).

Objective: To describe the electrophysiological features of the JJR in patients with early-stage ALS.

Methods: We studied 17 patients with a definitive diagnosis of bulbar onset ALS (<1 year of evolution) and 15 age-matched healthy controls. The reflex was evoked by tapping the subject's chin at rest with a modified neurologist's reflex hammer that also triggered the oscilloscope recordings. The responses were recorded with surface electrodes placed over the masseter muscle. All subjects were stimulated 20 times, at intervals of 30 seconds. Average onset latency and peak to peak amplitude, as well as the percentage of occurrence of the responses, were measured in all subjects.

Results: ALS patients showed significantly increased JJR amplitudes compared to controls (370 ± 65 uv. vs. 250 ± 110 uv.), $p < 0.05$). The percentage of occurrence was also significantly higher in patients than in normal subjects (75.5 ± 17.3% vs. 55.5 ± 18.2%, $p < 0.05$) and ALS patients showed a poor habituation of the responses over repeated tapings. Average onset latency was reduced in the ALS group, as compared to controls, but this difference was not statistically significant (6.7 ± 1.5 ms. vs. 7.3 ± 1.3 ms, $p > 0.05$).

Conclusion: These findings demonstrate that patients with early stage ALS have a facilitated JJR. These enhancement could be explained by two reasons: 1- Increased suprasegmental excitatory influences on brainstem motor circuits. 2- Decreased cortico-bulbar inhibitory influences on brainstem motor neurons, as happen with the other myotatic reflexes. The study of JJR could provide evidence of UMN involvement, independent of cervical spinal cord compression, which could be helpful to support an earlier ALS diagnosis.

C53 QUANTITATIVE MUSCLE ULTRASONOGRAPHY IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: early diagnosis, muscle ultrasonography, quantitative analysis

Background: Amyotrophic lateral sclerosis (ALS) can be difficult to diagnose, especially in an early stage of the disease. Quantitative muscle ultrasonography can be used to detect structural muscle changes in neuromuscular diseases. Furthermore ultrasonography can detect movements like fasciculations.

Objectives: The primary objective is to examine whether quantitative muscle ultrasonography can detect structural muscle changes in early-stage ALS. In addition a longitudinal study was performed to asses if quantitative muscle ultrasonography can be used to monitor disease progression.

Methods: Bilateral transverse scans were made of five muscles or muscle groups (sternocleidomastoid, biceps brachii/brachialis, forearm flexor group, quadriceps femoris and anterior tibial muscles) in 75 patients suspected of having ALS. Echo intensity and muscle thickness were determined for each muscle. Twenty-five patients were also screened for fasciculations. For the longitudinal study 35 patients were examined five times with an interval of six weeks using the previously described ultrasound protocol. In addition the ALSFRS was administered and muscle strength was tested (manual muscle testing) each time.

Results: Of the 75 patients included, 48 patients received a final diagnosis of ALS and were analyzed further. Quantitative analysis revealed a significant increase in echo intensity in all muscles and a decrease in muscle thickness of the biceps brachii, forearm flexors and quadriceps femoris on both sides. Fasciculations were easy to detect in multiple muscles of all screened patients except one. The results of the follow up study will be presented at the Symposium.

Discussion and Conclusion: Quantitative ultrasound can be used to detect muscle changes, including fasciculations, caused by ALS in an early phase of the disease. Our results suggest that quantitative muscle ultrasonography probably can be used to increase diagnostic certainty by detecting muscle abnormalities in clinically unaffected regions. Particularly now a more prominent role for fasciculations in the electrophysiological diagnosis of ALS is suggested (1).

The results of the longitudinal study are needed to verify if quantitative muscle ultrasonography can also be used to monitor disease progression.

Reference:

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C54 USING THE CLINICAL PHENOTYPE TO PREDICT MRI CHANGES IN ALS: A 4 TESLA STUDY USING FRACTIONAL ANISOTROPY

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Keywords: diffusion tensor imaging, corticospinal tracts, phenotypes

Background: Several diffusion tensor imaging (DTI) studies of the corticospinal tracts (CST) in ALS have demonstrated reduced fractional anisotropy compared to controls. Still, exact correlations between the motor disability and the severity of diffusion abnormalities remain uncertain. Several studies have explored the relationship of FA findings to the severity of motor signs and have found correlations, yet it is not clear whether specific patterns of motor involvement can predict these abnormalities.

Objectives: Using DTI and recording fractional anisotropy (FA), we sought to determine if patients with ALS that involved only the limbs differed from those with generalized patterns within the CST or in other frontal regions.

Methods: We created a clinical categorization scheme that divided patients into two basic phenotypes. We defined generalized ALS (GALS) in patients who had upper motor neuron involvement in the bulbar region and at least one limb region, and had lower motor neuron involvement in at least one limb at the time of study. Localized ALS (LALS) was defined as upper motor neuron and lower motor neuron involvement only in the limbs at the time of the study. Eighteen patients and fifteen controls underwent DTI imaging using a 4.0-Tesla (Bruker/Siemens) MRI system, based on EPI sequence (TR/TE = 6000/77ms; field of view 256 × 224cm; 128 × 112 matrix size, 2 × 2 × 3 mm^3 resolution; b = 0, and b = 1000 with 6 non-collinear directions). Alignment of FA images from all study subjects was performed by SPM2 software. Analyses were performed in ALS versus controls and between generalized and regional ALS voxel-by-voxel accounting for age and gender. Significance level was set at $p < 0.005$ without controlling for multiple comparison.

Results: The mean age of the ALS cohort was 58 years, mean FVC 91% and mean ALSFRS-R score 35. Patients with GALS had a shorter duration of disease (mean 17.5 months v 32.8), lower FVC (mean 82.5% v. 98.2%) and lower ALS FRS score (33 v. 37). Compared to controls, there was significant FA reduction along motor fibers and across the corpus callosum in GALS, but only minimal change in the motor fibers in LALS versus controls. For the overall cohort, ALSFRS-R positively correlated with FA in bilateral motor fibers, frontal WM, temporal white matter but we did not see strong correlations with duration of disease or FVC.

Discussion and Conclusions: A correlation between FA along the CST and ALS-FRS is established from previous studies. However, our findings also suggest that the clinical pattern can help predict whether CST involvement will be present in the brain by FA. In particular, upper motor neuron bulbar involvement, which was the primary method to differentiate patients in this study, were associated with greater CST and colossal involvement, while limb-only patterns did not show the extent or degree of these central changes. The findings give some indication that the spread and degree of disease centrally differs between the varied phenotypes.

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SESSION 8A GENETICS

C55 GENOME WIDE ASSOCIATION STUDIES IN COMPLEX DISEASES: LESSONS FOR ALS

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No abstract received.

C56 WHOLE GENOME ASSOCIATION STUDY REVEALS GENETIC VARIANTS THAT MODIFY SURVIVAL IN SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: Amyotrophic Lateral Sclerosis; SNP; Whole Genome Association Study

Background: Amyotrophic lateral sclerosis is a degenerative disorder of motor neurons that typically develops in the sixth decade and is uniformly fatal, usually within five years. About 10% of ALS cases are dominantly inherited whereas sporadic ALS is thought to be multifactorial, with environmental, infectious and genetic etiologies.

Objectives: To identify genetic variants associated with susceptibility and phenotypes in sporadic ALS, we performed a genome-wide analysis of single nucleotide polymorphisms in ALS cases and controls.

Methods: In a set of 1829 sporadic ALS (SALS) cases and 2250 controls, we completed an unbiased analysis of ~288,357 SNPs (1,176,208,203 genotypes) distributed across the genome. Genotypes were obtained from three sources in the USA (total 917 ALS, 912 controls) and three in Europe (904 ALS, 1346 controls) and analyzed as a single set of SALS cases and controls. Genotypes were used for the analysis of four phenotypes in SALS: susceptibility, site of onset, age of onset and survival of disease.

Results: From our study, we identified a SNP that yielded a genome-wide significant result that withstood Bonferroni correction (uncorrected and corrected p values of 1.84E-08 and 0.021) for association with survival (n = 1014). Homozygosity for the favorable allele conferred a 14.0 months survival advantage. No SNPs were significantly associated with risk of sporadic ALS, site of onset, or age of onset.

Conclusions: These findings support the view that genetic factors modify survival in ALS. In our view, the identification of a potential determinant of rate of progression of sporadic ALS is therefore promising; insights into this molecular pathway may provide new targets for therapies to slow this devastating disease.

C57 SCREENING FOR REPLICATION OF GENOME-WIDE ASSOCIATION SIGNALS IN THE IRISH AND POLISH ALS POPULATIONS

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Keywords: Genome wide study, DPP6, genetics

Background and Objectives: We recently reported a joint analysis of genome-wide data on 958 sporadic ALS (SALS) cases and 932 controls from Ireland and publicly available datasets from The Netherlands and US. The strongest pooled association was rs10260404 in the DPP6 gene. Here, we sought replication of joint analysis signals in both an expanded Irish and a Polish SALS cohort.

Methods: The study populations comprised an expanded Irish cohort (312 patients with SALS; 259 controls) and an additional Polish cohort (218 patients; 356 controls). Among 287,522 autosomal SNPs, 27 were commonly associated for the same allele at a p value below 0.1 in the Irish and US and below 0.05 in the larger Dutch set. These 27 SNPs were genotyped using KASPar assays. Allelic p values were computed using standard chi-squared statistics in PLINK.

Results: Eleven SNPs, including rs10260404, reached a final p value below 0.05 in the Irish. None of these showed association with SALS in the Polish cohort. Pooling of data from this study and the previous genome scans (1267 SALS; 1336 controls) did not identify any association reaching Bonferroni significance ($p < 1.74 \times 10^{-7}$) or of stronger significance than pooling the genome-wide data alone.

Conclusions: The present strategy did not reveal any consistently associated SNP across four populations. The results may be explained by population-specific differences or by the relatively low power of each included dataset. Future expansion of genome-wide SNP data will refine susceptibility loci for SALS both within and between populations.

C58 CLINICAL AND GENETIC PHENOTYPE OF 283 ALS FAMILIES

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Keywords: Familial ALS, Phenotype, SOD1 mutations

Background: Amyotrophic Lateral Sclerosis is the most frequent motor neuron disorder in adults. Almost 10% to

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20% of ALS cases are familial (FALS). To date, in the literature, phenotypes of familial and sporadic forms are considered similar. However, it is frequently noted that FALS cases have an earlier age of onset and bulbar onset is rare. SOD1 mutations are supposed to account for at least 20% of FALS cases. However there are few large studies focused on phenotype analysis of FALS cases.

Objectives: To describe clinical and genetic phenotype of 283 French FALS, to determine whether they correspond to the model described in many studies: a dominant inheritance, an earlier onset, a rare bulbar onset and a longer duration.

Material and Methods: A FALS network has been developed in France since 1996 for collecting as exhaustively as possible the families in our country. To date, 283 families have been identified. We collected clinical characteristics: gender, age of onset, site of onset, ALS duration. For all the FALS cases a pedigree was drawn. When appropriate or needed we studied probands for SOD1 mutations. We separated, and then compared, the families according to the number of generations involved in the ALS process. In type I families (one generation), siblings or cousins had ALS. In type II, a parent and his offspring were affected (or uncle and nephew). In type III, ALS was distributed across three generations. We also compared, in type I and II, families with 2 ALS patients with those with more than two affected members (I+ and II+).

Results: The group of 283 families was composed of 71 type I, 181 type II and 31 type III.

In type I FALS with 2 affected members (n = 62), sex ratio was 0.7, mean disease onset was 58yrs, 32% had bulbar onset, median ALS duration was 24.5 months, 5.5% of families carried SOD1 mutations. In type I+ families (n = 9), sex ratio was 1.3, mean disease onset was 58yrs, 48% had bulbar onset, median ALS duration was 35 months, 25% of families carried SOD1 mutations.

In type II FALS with 2 affected members (n = 132), sex ratio was 0.9, mean disease onset was 58yrs, 32% had bulbar onset, median ALS duration was 30 months, 7% of families carried SOD1 mutations. In type II+ (n = 49), sex ratio was 1, mean disease onset was 54yrs, 30% had bulbar onset, median ALS duration was 25.5 months, 19% of families carried SOD1 mutations.

In type III FALS (n = 31), sex ratio was 1.1, mean disease onset was 49yrs, 12% had bulbar onset, median ALS duration was 30 months, 29% of families carried SOD1 mutations.

Discussion: A small part of the FALS pedigrees (11%) are undoubtedly consistent with a dominant transmission. Their clinical and genetic phenotype well fit with the "FALS model" described previously. The vast majority of FALS cases (type I and II with 2 affected members, n = 184, 65%) are consistent with a complex genetic trait. However, as SOD1 mutations may be encountered in all three types, the percentage of families with a low penetrance cannot be strictly estimated. Nevertheless it seems that this percentage is not significantly high. New strategies for studying FALS cases are warranted.

C59 *ANG* K17I MUTATION SEGREGATING WITH AUTOSOMAL DOMINANT FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS IN A LARGE DUTCH PEDIGREE

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Keywords: ANG, FALS, ALS-FTDP

Background: Using a candidate gene approach mutations in *ANG* were identified in both sporadic and familial ALS patients, but not in a large cohort of healthy controls. Subsequent screens in other populations have also identified *ANG* mutations in ALS patients. However, these studies also found mutations in healthy controls, suggesting that not all *ANG* mutations are pathogenic.

Objectives: A study of familial ALS in The Netherlands was performed to: i) determine incidence and prevalence of FALS in The Netherlands, ii) to determine pattern of inheritance of FALS in each pedigree. All families were screened for mutations in SOD1 and ANG.

Methods: 39 families with ALS were included in the study. The index patient from each family was screened for mutations ANG. ANG mutation analysis was performed in all subjects as follows: DNA was amplified with PCR using primers: ANG_xn2_For, 5′ TGTTCTTGGGTCTACCACAC; ANG_xn2_Rev, 5′ AATGGAAGGCAAGGACAGC. Both forward and reverse strands were sequenced with the same primers used for PCR amplification. Sequence reaction products were purified using Sephadex (GE Healthcare) columns and run on an ABI 3730 automated sequencer. Traces were analyzed using ContigExpress from the Vector NTI Suite10 (Invitrogen). The K17I mutation was confirmed by repeating all sequencing steps in each individual using newly made DNA solutions from stock. A total of 275 ethnically matched control subjects were also screened for *ANG* mutations.

Results: In 39 probands from families with ALS, one mutation was identified in one proband (K17I). Subsequent analysis of the pedigree revealed an autosomal dominant pattern of inheritance. In this large family, DNA was available from 44 individuals (3 additional affected family members). The K17I mutation segregates with disease in this pedigree and was not found in 275 unrelated, healthy

controls from The Netherlands. The I46V mutation was found one control.

Phenotypically, all affected family members had limb onset disease with rapid to average progression, respiratory involvement and minimal upper motor neuron signs. Interestingly, one patient had been diagnosed with Parkinson's disease 5 years prior to the onset of ALS and recently started to demonstrate altered behaviour, sexual disinhibition and problems performing complex tasks suggestive of FTD.

Discussion: Here, we present a large Dutch pedigree with an autosomal dominant form of familial ALS in which the K17I mutations clearly segregates with disease. Before *ANG* mutation screening is used diagnostically, as a target for drug therapy or transgenic animal models are created it is important to elucidate which mutations are pathogenic. This study provides evidence that the K17I mutation is pathogenic and may also be implicated in FTD and Parkinsonism.

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SESSION 8B COGNITIVE & PSYCHOLOGICAL CHANGE

C60 RAPID SCREEN EXAM FOR THE DETECTION OF FRONTAL AND TEMPORAL DYSFUNCTION SYNDROMES: APPLICATION TO ALS

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Keywords: Dementia, Cognition, Intervention

Background: The prevalence of frontotemporal dementia (FTD) in the ALS population is estimated to be between 30 to 50 percent. FTD is recognized to present as three distinct subtypes, including isolated behavioural change, a frontal cortical associated aphasia of a progressive non-fluent type and a temporal cortical associated aphasia of a fluent type characterized by loss of semantic knowledge. Given the aggressive course of the disease process, we wanted to identify ALS patients whose emerging FTD was rendering them less capable of independent decision-making. We developed an evaluation approach that included a behavioural inventory as well as screening measures of language change characteristic of either frontal dysfunction (FD) or temporal dysfunction (TD).

Objectives: To develop incidence rates of ALS-FD and ALS-TD. To refine our multifaceted program of patient and family support, in response to the presence of either FD or TD, to keep the affected patient as fully engaged in ongoing treatment decision making as possible, from the time of diagnosis to the terminal stage of the disease process.

Methods: We administered our 20-minute exam to 118 limb-onset and 80 bulbar-onset ALS patients. We evaluated the incidence of acquired change in fluency, abstract reasoning, judgement and behaviour. Assessment included the Neurobehavioral Cognitive Status Examination, letter fluency, category fluency, and the New Adult Reading Test (NART). The NART provided an estimated verbal IQ. Reading was screened by the BDAE Oral Reading and Reading Comprehension-Short Form. Changes in behaviour and personality from a caregiver perspective were assessed by the Frontal Behavioural Inventory (FBI). Oral word list generation was followed by a word list copy task, to control for bulbar effects.

Results: Significant deficiencies were found in the domains of fluency, abstract reasoning, judgment and behaviour in 25.3%, 17%, 29.5% and 2.4% of limb onset and 38.1%, 19.2%, 46.7%, and 4.3% of bulbar-onset patients. Predominant FBI traits included logopenia, apathy, inflexibility, irritability, and verbal apraxia.

Discussion and Conclusions: Among patients with deficiencies, we found a predominance of cognitive frontotemporal dementia and temporal dementia, with only rare instances of isolated behavioural disturbance. The present study bridges the gap in screen evaluation normative studies of FTD in ALS. The sample was large in size, with strong representation of both non-bulbar and bulbar onset subtypes. The measures chosen allowed for detection and differentiation of both frontal and temporal dysfunction syndromes, as well as for the establishment of incidence rates of the three recognized FTD sub-types. The availability of a concise, practical FTD syndrome assessment tool in the multidisciplinary ALS clinic will strengthen a team's ability to recognize and address barriers to communication and decision making in the course of treatment planning, from the time of diagnosis to the terminal stage of the disease process.

C61 NEUROANATOMICAL CORRELATES OF APATHY IN ALS: A 4 TESLA STUDY USING FRACTIONAL ANISOTROPY.

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Keywords: Apathy, FTD, Imaging

Background: The association between ALS and frontotemporal dementia (FTD) has led to the study of behavioural abnormalities in ALS to better specify the prevalence of FTD in this population. The most common behavioural abnormality identified in ALS is apathy, although clarification is needed as to whether this reflects a frontally-mediated disorder versus the sequalae of factors such as respiratory compromise, weakness or psychological factors. In FTD studies (non-ALS) using voxel-based morphometry, apathy correlates with right hemisphere dysfunction and anterior cingulate changes. To better clarify whether a frontotemporal disease process may explain behavioural changes in ALS, we examined whether similar correlations exist in the ALS population with regards to apathy.

Objectives: Using a diffusion tensor imaging (DTI) method by measuring fractional anisotropy (FA), we used a voxel-based analysis of whole brain changes to investigate the loss of white matter integrity as it relates to apathy in ALS.

Methods: Eighteen non-demented patients with ALS were enrolled in an imaging study. DTI imaging was accomplished using a 4.0-Tesla (Bruker/Siemens) MRI system, based on EPI sequence (TR/TE = 6000/ 77ms; field of view 256×224cm; 128×112 matrix size, $2 \times 2 \times 3$ mm^3 resolution; $b = 0$, and $b = 1000$ with 6 non-collinear directions). Alignment of FA images from all study subjects was performed by SPM2 software. Correlation between FA and apathy scores was tested voxel-by-voxel using a general linear model accounting for age and gender. Significance level was set at $p < 0.005$ without multiple comparison. Neuropsychological evaluations were completed; apathy was assessed using the Frontal Systems Behavioral Scale. Patients with dementia or depression were excluded from the analysis.

Results: The mean age of the cohort was 58 years, mean FVC was 91% and mean ALSFRS-R score was 35. FA reduction along bilateral motor, temporal and frontal white matter, which included bilateral uncinate fibers and right anterior cingulate fibers, was significantly correlated with current apathy scores. When premorbid levels of apathy were controlled for, FA reduction was significantly correlated with alterations in bilateral superior frontal white matter and temporal-parietal white matter fibers.

Discussion and Conclusions: These results represent the first known dataset regarding neuroanatomical correlates of

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behavioural change in non-demented ALS patients. Findings suggest an organic basis for apathy and are consistent with research on apathy in FTD and other dementias. Frontal and temporal white matter damage may be associated with increased apathy, even early in the ALS disease course. Specifically, white matter changes in right hemisphere and anterior cingulate may be associated with apathy, consistent with previous findings in FTD.

C62 SOCIAL AND EMOTIONAL COGNITION AND BEHAVIOUR: EVIDENCE OF SUBCLINICAL FRONTOTEMPORAL DEMENTIA IN ALS

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Keywords: Cognition FTD Behaviour

Background: Although an association between ALS and frontotemporal dementia (FTD) has been recognized, the exact nature of the relationship has remained elusive. It is well established that a subgroup of ALS patients suffer from behaviour and cognitive change as found in FTD and a further proportion are thought to display more subtle cognitive change, indicating a continuum of impairment. Evidence in support of the association rests primarily on the repeated demonstration of verbal fluency impairments (a test of executive function sensitive to frontal lobe lesions) in non-demented ALS patients, but deficits on other tests have been less striking. FTD is a heterogeneous disorder and recent research has demonstrated that patients with frontal variant FTD may retain intact performance on standard neuropsychological tests at the early stages of the disease, despite a distinctive behavioural syndrome. Moreover these patients show early dysfunction in social cognition, emotional processing and decision making on experimental measures long before the onset of overt executive dysfunction.

Objectives: These studies investigated whether ALS patients show deficits on a range of experimental tests of social and emotional cognition and corresponding behaviour change similar to that found in the early stages of frontal variant FTD.

Methods: In the first study 19 patients with classical ALS and 20 healthy controls undertook a test of emotional decision making in which choices were made based on reward. Behavioural measures included the Frontal Systems Behaviour Scale and the Manchester Behaviour Interview. In the second study a group of 15 patients with ALS and 21 controls undertook a range of tests of social cognition (including judgement of preference based on eye gaze and reading the mind in the eyes) and tests of emotional cognition (recognition of emotions from facial expressions).

Results: Analysis of the emotional decision making test revealed a deficit in ALS patients, in that although controls learnt to avoid the disadvantageous stimuli through the task, the ALS patients did not. In addition, patients showed corresponding behaviour change (apathy, disinhibition and executive dysfunction) from the onset of the disease, with particular increases in apathy and changes in affect and social behaviour. In the second study, deficits were found on tests of social and emotional cognition. ALS patients were particularly impaired at inferring the mental state of others and in recognising other people's emotions.

Conclusions: These studies demonstrated evidence of deficits in social and emotional cognition and corresponding behaviour change on tests which have been shown to be sensitive to the early stages of frontal variant FTD. The findings indicate evidence of a subclinical syndrome in a significant proportion of ALS patients without overt dementia. The relative contribution of dorsolateral and ventromedial prefrontal pathways to the cognitive and behavioural profile in ALS are discussed.

C63 INFLUENCE OF FRONTOTEMPORAL DEMENTIA ON ALS PATIENTS AND CAREGIVERS QUALITY OF LIFE AND DEPRESSION

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Keywords: Frontotemporal dementia, quality of life, caregivers

Background: The frequency of frontotemporal dementia (FTD) in ALS patients ranges from 5 to 50% of cases, according to the methods used to diagnose cognitive deficits. The relationship between frontotemporal dementia and quality of life (QoL) in ALS patients and caregivers also remains unclear

Objective: To evaluate the frequency of frontotemporal dementia in ALS using the Frontal System Behaviour Scale (FrSBE), an instrument that evaluates patients' behavioural changes from the patient's and caregivers perspective, using three different scales: apathy, disinhibition, and executive functions; to evaluate the relationship between FTD and patients' and caregivers' QoL and depression.

Methods: Sixty-three consecutive ALS patients and their primary (unpaid) caregivers were administered the FrSBE, the Zung Depression Scale (ZDS), the McGill QoL Questionnaire (MQOL). Statistical analysis was performed with SPSS 12.0.

Results: Patients' mean age was 61.7 years (SD 10.3); 50.7% of patients were men. Caregivers mean age was 53.7 years (SD 12.7). Patients mean MQOL score was 6.8 (SD 1.3), ZDS score was 29.2 (SD 6.3); 11 patients (17.4%) were over the depression cut-off. Twenty-four (38.0%) patients showed emotional lability. Caregivers mean MQOL score was 6.7 (SD 1.3), mean ZDS score was 38.6 (SD 7.9). According to FrSCE, caregivers suspected that 39.8% of patients were over the FTD cut-off, while only 20.6% of patients reported a score over the cut-off for FTD. Considering the 3 subscales of the FrSBE, according to the caregivers evaluation no FTD component was prevalent, while patients reported themselves as significantly less disinhibited ($p < 0.01$) and with less executive deficit ($p < 0.01$) than caregivers did. Patients self-evaluating themselves over the cut-off for FTD had higher score for depression ($p < 0.01$) and lower scores for QoL ($p < 0.05$). Caregivers who reported patients' score over the cut-off for FTD had a lower QoL ($p < 0.01$) and higher depression ($p < 0.05$).

Conclusions: Using the FrSBE, 40% of patients evaluated by their caregivers had a score over the cut-off for FTD. FTD in ALS patients negatively influenced their caregivers' QoL and depression. Patients who reported a FrSBE score over the cut-off for FTD were more depressed and had a lower QoL than the other patients.

C64 RESEARCH INTO COGNITIVE CHANGE IN ALS/MND: IMPLICATIONS FOR CLINICAL CARE AND MANAGEMENT

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Keywords: cognition, behaviour, care

Research into cognitive change in ALS/MND has progressed considerably and it is now well-accepted that cognitive functions may be compromised in people with ALS/MND. However, a number of differences in approach to measuring and classifying cognitive change in the disease have resulted in conflicting views as to the prevalence of cognitive change and whether or not it may be progressive. In addition, a number of clinical variables may influence cognitive functioning and attention needs to be paid to these in understanding the nature or extent of cognitive change that may be found. New lines of research into cognitive change are also considering alterations in emotional processing in people with ALS/MND. Of related importance, research is being undertaken increasingly into the behavioural as well as cognitive manifestations of the disease process, again with differing approaches being taken to measurement and classification.

In addition to highlighting the profile of cognitive change in MND, the present talk will provide an overview of some of the issues and controversies concerning cognitive (and behavioural) involvement in the disease. Allowing for these, the practical implications of understanding how the potential nature of cognitive change in ALS/MND might be relevant to everyday decision-making and care will be discussed.

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SESSION 8C BEYOND GUAM: NEW ASPECTS OF THE CYNOBACTERIA/BMAA HYPOTHESIS

C65 BMAA AS A POSSIBLE TRIGGER FOR SPORADIC ALS/MND: INSIGHTS FROM THE CHAMORRO

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Keywords: BMAA, Guam, Neurotoxic Diet

Background: The hypothesis that the neurotoxic amino acid BMAA can trigger sporadic neurodegenerative illness in vulnerable individuals depends on four pillars: 1) BMAA is produced by cyanobacteria, which are ubiquitous throughout the world; 2) in the known ALS/PDC foci, exposure to high levels of BMAA can result from biomagnification and dietary ingestion from multiple sources, while in other areas, low-level exposure occurs is occurs through cyanobacterially-contaminated water supplies (1); 3) vulnerable individuals accumulate BMAA in their neuroproteins (2), and 4) accumulation of BMAA in neuroproteins can trigger neurodegenerative illness.

Objectives: To determine if dietary exposure to BMAA among the Chamorro is associated with increased risk of ALS/PDC.

Methods: We interviewed 23 Chamorro villagers of varying ages from Umatac and Merizo villages after they signed an informed consent form. A code number was attached to a small locket of hair that each villager supplied and then the sample was blinded for BMAA analysis. BMAA was detected by protein hydrolysis and AQC-derivatization in a reverse phase HPLC-FD system.

Results: We found a significant relationship between disease and level of flying fox consumption ($\chi^2 = 3.86$, $p < 0.05$) with a highly significant relationship ($\chi^2 = 26$; $p < 0.001$) between disease status and moderate to high flying fox consumption. However, we found no significant relationship ($\chi^2 = 0.91$, NS) between flying fox consumption and the presence of BMAA in hair, since many villagers who had not consumed flying foxes still had detectable BMAA. Only small children who had not consumed possible BMAA sources (flying foxes, pigs, deer, land crabs, or cycad flour) had no detectable BMAA in their hair.

Discussion: These results support previous findings that protein-bound BMAA in cycad flour and feral animals that feed on cycad seeds result in significant BMAA inputs into the Chamorro diet (3). The results of Borenstein *et al.* (4) also support the suggestion that protein-bound BMAA from multiple sources may contribute to neurological disease. Although they did not find a specific correlation linking disease state with flying fox consumption, they did find widespread consumption of flying foxes among Chamorros aged 65 and older and statistically significant correlations with disease state and cycad consumption. This suggests a broad exposure of the Chamorro population to dietary BMAA, which may serve as a trigger for neurodegenerative illness.

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C66 CYANOBACTERIA, NEUROTOXICITY AND WATER RESOURCES

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Keywords: Cyanobacteria, BMAA, neurotoxicity

Background: Cyanobacteria are ancient, photosynthetic bacteria that occur throughout the world in a wide range of environments. They are most commonly known for their ability to form mass occurrences in waterbodies used for recreation and the preparation of human drinking water. In addition to their effects on the aesthetic quality of waterbodies, cyanobacteria are capable of producing a wide range of bioactive compounds, including potent hepato- and neurotoxins. Although the majority of the neurotoxins are acutely toxic, little is known concerning their long-term health effects. Included in these is β-*N*-methylamino-L-alanine (BMAA) which has been associated with an Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex (ALS-PDC) in Guam, thought to accumulate through the diet.

Objectives: The purpose of the study was to investigate the occurrence of BMAA in environmental cyanobacterial bloom material from waterbodies in the UK. The waterbodies selected all have a history of either being used for the preparation of human and/or animal drinking water, for recreation or as fisheries and are sites of known animal poisoning incidents as a result of cyanobacterial blooms.

Methods: Cyanobacterial bloom material collected from 12 waterbodies was examined for the presence of cyanobacteria by microscopy. Material was lyophilised and extracted to analyse for anatoxin-a, saxitoxins and microcystins using mouse bioassay, high performance liquid chromatography (HPLC) and ELISA. BMAA was derivatised and determined by HPLC with fluorescence detection and LC-MS/MS as either a free amino acid or associated with a precipitable protein fraction.

Results: Bloom samples were found to contain cyanotoxins, and in the case of acute animal intoxication, cyanotoxins were considered to be the proximal cause. With respect to BMAA, all 12 samples contained BMAA as either a free amino acid and/or associated with precipitable protein at between 2 and 275μg g^{-1} dry weight of material.

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Discussion and Conclusions: BMAA was found to co-occur with known cyanotoxins in cyanobacterial blooms from UK waterbodies. Although animal intoxications occurred at these sites, cyanotoxins other than BMAA were considered to be the cause of the mortalities. However, the results suggest that BMAA may occur in the environment and in places where humans are potentially exposed to this amino acid. Due to its associations with human neurological disease, further assessment of BMAA in the environment is required.

C67 PRODUCTION OF THE NEUROTOXIN BMAA BY CYANOBACTERIA THROUGHOUT THE WORLD: IMPLICATIONS FOR HUMAN HEALTH

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Keywords: Sporadic ALS, Cyanobacteria, Environmental Neurotoxins

Background: BMAA (β-N-methylamino-l-alanine) is putatively linked to ALS/PDC (1). Early reports concluded that washed cycad flour contains extremely low levels of BMAA (2). However, they failed to analyze the protein fraction of this flour that contains up to 169 μg/g of BMAA (3). BMAA is produced by symbiotic cyanobacteria present in the cycad roots. BMAA is now known to be produced by cyanobacteria throughout the world (4, 5).

Objectives: We sought to determine if free-living species of the genus *Nostoc* (6) from around the world produce BMAA and if preservation methods affect detection in paired frozen or fluid preserved human tissues.

Methods: We used 5 different analytical methods, HPLC-FD, Amino Acid Analyzer, UPLC-UV, UPLC/MS, and triple quadrupole LC/MS/MS to detect BMAA in *Nostoc* and frozen and fluid human tissues.

Results: All five methods detect BMAA in free-living *Nostoc*. Fluid fixation of human tissues results in an underestimate of total BMAA.

Conclusions: Detection of BMAA at low concentrations in a complex physiological matrix, is best accomplished with multiple methods of detection. Verification using LC/MS/MS is definitive. Since low concentrations of BMAA (10–30 μM) kill motor neurons (7, 8), our demonstration that free-living *Nostoc* produces BMAA supports the suggestion that human exposure to low concentrations of BMAA can occur throughout the world (9). To test whether such exposure is associated with sporadic ALS/MND will require further epidemiological studies.

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C68 *IN VITRO* NEUROTOXICITY OF THE CYCAD TOXIN, BETA-METHYLAMINO-L-ALANINE (BMAA)

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Keywords: BMAA, motor neuron, neurotoxicity

Background: The cycad toxin, beta-methylamino-L-alanine (BMAA) was first proposed as a contributor to the amyotrophic lateral sclerosis-Parkinsonism dementia complex of Guam (ALS/PDC) based on the ability of large amounts of this compound to induce a similar disease phenotype in primates. However, concerns about the apparent low potency of this toxin in relation to estimated levels of human ingestion led to doubts about its disease relevance.

Objectives: We have examined the in vitro neurotoxicity of BMAA in order to gain insights into both its mechanisms of toxicity and potency as a neurotoxin.

Methods: Neurotoxicity and imaging studies were carried out on mixed cortical or spinal neuronal cultures.

Results: Although BMAA had reported to induce excitotoxic neuronal injury, largely via activation of NMDA receptors, in our early studies we found that BMAA had no direct excitatory effects unless the exposure was carried out in the present of bicarbonate. We and others concluded that in the presence of bicarbonate, a carbamate adduct of the side chain amino group of BMAA results in a structure resembling glutamate and capable of activating glutamate receptors (1,2). In further studies we found evidence that although BMAA appeared to be a fairly weak NMDA receptor agonist, low levels of BMAA could selectively damage vulnerable subpopulations of neurons via activation of AMPA/kainate type glutamate receptors (3), leading us to hypothesize that these receptors might be of particular importance to motor neuronal (MN) degeneration, an idea supported by a large number of intervening studies. More recently, we have used mixed spinal neuronal cultures to examine the specific vulnerability of MNs to BMAA. We found that BMAA induced selective MN loss at concentrations ($\sim$30 μM) which were significantly lower than those causing widespread neuronal degeneration (4). Furthermore, this MN injury was blocked by the selective AMPA/kainate receptor antagonist, NBQX. Using imaging techniques, we further found that BMAA induced preferential $(Ca^{2+})_i$ rises and selective reactive oxygen species (ROS) generation in MNs with minimal effect on other spinal neurons.

Discussion and Conclusions: Recent studies have identified new and diverse environmental sources of BMAA and have found evidence that BMAA can be incorporated into proteins, possibly providing an endogenous reservoir from which it can be slowly released (5). Thus, in light of our *in vitro* studies, we propose that protein bound BMAA *in vivo* might contribute to slow MN injury in part via release from proteins, and formation of carbamate adducts, causing activation of AMPA/kainate type glutamate receptors.

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C69 NEW ASPECTS OF THE CYANOBACTERIA/ BMAA HYPOTHESIS AND FUTUREDIRECTIONS

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Keywords: Cyanobacteria, BMAA, sporadic ALS

Background: The cause of ALS in the 85% of cases with sporadic disease is unknown and presumably environmental. Even in the 15% of familial cases there must be an environmental factor that precipitates the onset. Evidence is compelling that a cyanobacterial product, probably BMAA, in cycads causes Guamanian ALS/PDC. BMAA is a non-natural neurotoxic amino acid that becomes incorporated into the proteins of higher organisms. Protein-bound BMAA has been found in millimolar concentrations in brains of Chamorros dying from ALS/PDC and North American ALS and Alzheimer's disease patients, but not in control brains. Cyanobacteria that produce BMAA and other neurotoxins are ubiquitous, particularly in water resources associated with animal deaths.

Objectives: To discuss: 1) How BMAA is incorporated into brain proteins; 2) How BMAA might cause neurodegeneration; 3) Testable predictions of the cyanobacteria/BMAA hypothesis; and 4) Therapeutic predictions derived from the hypothesis.

Discussion: C.A.Shaw and colleagues have argued that steryl glucosides produced by cyanobacteria and other organisms are the cause of Guamanian ALS/PDC. Their hypothesis would suggest that BMAA is simply a marker of exposure to cyanobacteria. However, several *in-vitro* and *in-vivo* studies have demonstrated that BMAA is neurotoxic at concentrations comparable to that found in human brains, though most were studies of acute toxicity. No true animal model of BMAA chronic neurodegeneration (developing after several months of feeding) has yet been reported.

BMAA is transported across the blood-brain barrier via the high affinity saturatable L1 system that carries large neutral essential amino acids (LNEAA-leucine, valine, methionine, histidine, iso-leucine, tryptophan, phenylalanine, threonine). These LNEAA are actively exported from the brain by several Na^+-dependent amino acid transporters to maintain a CSF concentration 10% of that of plasma. It is not known whether BMAA is actively exported across the blood-brain barrier, or which tRNA is responsible for incorporating BMAA into protein. The metabolic pathways for BMAA are not known. More basic information about BMAA is required.

The cyanobacteria/BMAA hypothesis predicts that blocking access of BMAA to the brain would prevent neurodegeneration. At least five trials of branch-chain amino acids in ALS have been reported; one showed benefit, one worsening, and three no effect. The dose required to saturate the L1-system and inhibit entry of BMAA is approximately six-times that used in these trials. Studies of ultra-high-doses of LNEAAs in ALS may be indicated. However, such treatment would be ineffective in patients already suffering from ALS unless BMAA is a recycling neurotoxin, as Murch and colleagues hypothesized, and unless high-dose LNEAA promotes the efflux of BMAA.

Conclusions: The cyanobacteria/BMAA hypothesis is an important advance in identifying the cause of sporadic ALS and the precipitation of familial ALS. Research to understand more about the association of cyanobacteria with ALS, the metabolism of BMAA and the production of models of chronic neurodegeneration may lead to a breakthrough in our understanding of ALS.

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SESSION 9A IN VIVO MODELS

C70 SMN DEFICIENCY ACCELERATES PROGRESSION IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: survival motor neuron, spinal muscular atrophy, SOD1

Background: SMA and familial ALS are fatal motor neuron diseases resulting from mutations in *SMN* and *SOD1* respectively. *SMN* genotypes predicting lower SMN protein levels are associated with increased risk and severity of sporadic ALS, suggesting a potential modulating role. Furthermore, a major genetic modifying locus encompassing the *SMN* region is implicated in the delayed phenotype of transgenic familial ALS mice on different background strains. Thus, several lines of evidence suggest possible genetic or molecular interactions between *SOD1* and *SMN* in ALS.

Objectives: To investigate SMN expression in familial ALS models and assess the impact of *SMN* genetic deficiency on the clinical phenotype and neuropathology of transgenic $SOD1^{G93A}$ mice.

Methods: SMN expression was analysed in stably transfected NSC34 cell lines and spinal cords of transgenic mice expressing SOD1 mutations using quantitative PCR and immunoblotting. Transgenic $SOD1^{G93A}$ mice were crossed with *SMN* heterozygous knockout mice and examined for motor function, survival and neuropathology. In addition, SMA type I mice were bred onto a background of wild-type SOD1 overexpression to determine rescue.

Results: ALS-linked mutant SOD1 expression depleted SMN protein levels in cellular and mouse models with significant downregulation from presymptomatic disease (60 days). Accordingly, genetic disruption of *SMN* significantly worsened rotarod performance and survival in transgenic $SOD1^{G93A}$ mice. In contrast, transgenic elevation of normal SOD1 in SMA mice failed to modify severity, suggesting an interaction specifically between mutant SOD1 and SMN.

Conclusions: These results establish that genetic reduction of *SMN* enhances phenotypic severity in transgenic familial ALS mice, supporting association studies where reduced *SMN* gene copy number predisposed to sporadic ALS. Thus, *SMN* reduction may be an enhancing genetic modifier of ALS. Secondly, SMN protein was progressively diminished in spinal cords of transgenic ALS mice, suggesting a relationship to lower motor neuron degeneration. We therefore propose that SMN replacement and upregulation strategies being developed as therapies for SMA may have potential benefit in patients with ALS.

C71 A NOVEL MUTATION IN GLYCINE TRNA SYNTHETASE AMELIORATES SOD1G93A MOTOR NEURON DEGENERATION.

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Keywords: glycine tRNA synthetase, Gars, SOD1G93A

Background: We are studying several novel mouse mutants potentially with motor neuron degeneration in order to characterise their phenotypes and further our understanding of human motor neuron disease.

Objectives: We have positionally cloned and characterised (with a PhD studentship from the UK Motor Neurone Disease Association and other funding) a new mouse mutant that has a defect in the glycine tRNA synthetase gene, Gars. Mutations in the human Gars gene results in a range of clinical manifestations from Charcot-Marie-Tooth disease to severe infantile muscular atrophy. We have characterised this mouse and have gone on to cross it to SOD1G93A transgenics.

Methods: We have undertaken a range of studies (paper submitted) of our Gars mice and proceeded to produce Gars,SOD1G93A double mutant; these have been characterised for lifespan and physiological and histological status.

Results: Our mouse has a dominant mutation resulting in loss of grip strength and sensory deficits. When crossed to SOD1G93A animals, the Gars,SOD1G93A double mutants have a striking and significant extension of lifespan and show delay in the histological characteristics of motor neuron disease compared to their SOD1G93A littermates.

Discussion and Conclusions: Suprisingly, the SOD1G93A, Gars double mutants have a significantly increased lifespan compared to their SOD1G93A littermates. This extension of lifespan is, from current data, even more than the ~20–28% shown in another cross between SOD1G93A and Loa (Legs at odd angles) mice that we and Chen, Popko and colleagues have previously published. Both Gars and dynein are involved either directly or indirectly in local translation in axons and in neurite outgrowth, suggesting an interesting connection between them that may relate to the protection they offer from SOD1G93A induced motor neuron death.

C72 LOSS OF THE HSJ1 MOLECULAR CHAPERONE EXACERBATES DISEASE PHENOTYPE IN $SOD1^{G93A}$ MICE

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Keywords: SOD1, HSJ1, chaperone

Background: The $SOD1^{G93A+/-}$ mouse and $SOD1^{G93A+/-}$ transfected cell cultures are commonly used models of ALS. Although the precise pathogenesis of ALS remains unclear, several lines of evidence now indicate that molecular chaperones play a role in this disease. For example, SOD1 mutants have been shown to alter the activity of molecular chaperones.

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Molecular chaperones facilitate refolding or proteolysis of mutant or damaged proteins. Therefore, a reduction in the activity of molecular chaperones will affect the ability of motor neurons to defend themselves against the toxic properties of mutant SOD1.

Human HSJ1 proteins are neuronal members of the DnaJ family of molecular chaperones. Recent studies have indicated that over-expression of HSJ1 proteins, both in vitro and in vivo, provide beneficial effects on various pathological states associated with protein misfolding and protein aggregation. For example HSJ1a has been shown to reduce protein aggregation associated with polyglutamine expansions in Huntington's disease and SMBA. (1,2). In this study we examined the role of HSJ1 proteins in the $SOD1^{G93A}$ mouse model of ALS.

Objective: To investigate the effect of manipulating the HSJ1 gene on disease progression in $SOD1^{G93A}$ mice crossed to HSJ1 knockout mice.

Methods: HSJ1 knock-out (KO) mice were produced by homologous recombination. $SOD1^{G93A+/-}$ male mice were mated to $HSJ1^{+/-}$ females, and the offspring were back crossed in order to produce homozygous knockouts, both with and without the $SOD1^{G93A+/-}$ mutation.

This cross resulted in progeny of 4 different genotypes: SOD1; WT; $HSJ1^{-/-}$ and $HSJ1^{-/-}$/SOD1. The effects of HSJ1 KO on disease progression was monitored by observation of behaviour and body weight. At 120 days of age, mice of each genotype were anaesthetised and prepared for *in vivo* physiological analysis of hindlimb muscle force and motor unit survival, as previously described (3).

Results: $HSJ1^{-/-}$ mice were healthy, viable and fertile. At 120 days of age, we observed no significant difference in weight or force of hind limb muscles between HSJ1 KO and WT mice. Although there was a reduction in muscle weight and force in SOD1 and $HSJ1^{-/-}$/SOD1 mice, there was no significant difference between these 2 groups. By 120 days of age, as previously reported, there is a significant reduction in the number of motor units in EDL muscles of SOD1 mice (ANOVA; $p < 0.001$). Importantly, in $HSJ1^{-/-}$/SOD1 mice, there was a significant reduction in motor unit survival compared to SOD1 mice ($p < 0.05$).

Discussion and Conclusions: These results suggest that HSJ1 may play an important role in motor neuron survival and indicate that the absence of HSJ1 chaperones in $HSJ1^{-/-}$/SOD1 mice increases the extent of motor neuron degeneration observed in SOD1 mice at 120 days. We are currently examining the mechanisms underlying these deficits and are investigating the effect of HSJ1 over-expression in SOD1 mice.

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C73 A COPPER-BIS (THIOSEMICARBAZONATO) COMPLEX DELAYS DISEASE PROGRESSION AND INCREASES SURVIVAL IN A TRANSGENIC $SOD1^{G93A}$ MOUSE MODEL OF ALS

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Keywords: transgenic SOD1G93A, matrix metalloprotease-9, metal complex

Background: Amyotrophic Lateral Sclerosis (ALS) is an adult-onset fatal neurodegenerative disorder that involves progressive deterioration of motor neurons. It is clinically manifested by weight loss, muscle wasting, and spasticity leading to paralysis and eventually death through respiratory failure. Although the aetiology of this debilitating disease remains unclear, more than 100 different mutations in the copper-zinc superoxide dismutase (SOD1) gene can cause familial ALS, implicating a role for SOD1 in ALS pathogenesis. Transgenic mice overexpressing human mutant $SOD1^{G93A}$ ($TgSOD1^{G93A}$) produce a phenotype that closely replicates both clinical and pathological hallmarks of human ALS. Mutations in SOD1 are believed to induce a gain in cytotoxic function causing degeneration of the motor neurons, possibly via oxidative stress. Diacetyl*bis*(N^4-methyl-3-thiosemicarbazonato) copper(II) (Cu(ATSM)) is a metal complex that crosses the BBB and has the potential to modify ALS through inhibition of oxidative stress.

Objectives: This study was carried out to evaluate the physical and biochemical effects of Cu(ATSM) treatment on the $TgSOD1^{G93A}$ murine model of ALS.

Methods: $TgSOD1^{G93A}$ mice in C57B6 background with delayed phenotype due to low copy of transgene were treated with Cu(ATSM) ($n = 14$) or vehicle ($n = 18$). Treatment was orally administered 5 days per week and commenced at the pre-symptom age of 140 days. Clinical assessment and motor function tests including rotarod and stride length were performed for all mice, and zymography was also performed to investigate matrix metalloprotease-9 (MMP-9) activity in spinal cord and serum samples.

Results: Disease onset was significantly delayed in $TgSOD1^{G93A}$ mice treated with Cu(ATSM) (mean onset age ($\pm$SEM) of 261 ± 5.4 days, compared to 241 ± 1.7 days for vehicle treated control mice, $p < 0.001$). Cu(ATSM) also extended the life span of $TgSOD1^{G93A}$ mice by 37 days (14%, $p < 0.0001$). MMP-9 activity, which decreases as disease symptoms develop, was significantly restored to pre-symptomatic levels in Cu(ATSM) treated $TgSOD1^{G93A}$ mice.

Discussion: Delayed development of ALS-like symptoms in Cu(ATSM) treated $TgSOD1^{G93A}$ mice indicates that Cu(ATSM) may prevent motor neuron deterioration caused

by the $SOD1^{G93A}$ mutation. The mechanism of action may involve the compound's potential to restore MMP-9 activity. Further studies will be carried out to investigate the effects of Cu(ATSM) on oxidative stress and motor neuron survival in these mice. This will help define the mechanisms of motor neuron degeneration in ALS, and will facilitate the development of new therapeutic strategies.

C74 *DROSOPHILA* AS A MODEL SYSTEM TO ELUCIDATE THE MOLECULAR MECHANISMS UNDERLYING MOTOR NEURON DISEASES

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Keywords: Drosophila, VAPB, Neurodegeneration

Background: Motor Neuron Diseases (MNDs) encompass a group of inherited neurodegenerative disorders characterized by selective dysfunction and death of motor neurons leading to spasticity, muscle atrophy and paralysis. In 2004, hVAPB (human VAMP-associated protein B) was shown to be the causative gene of a clinically diverse group of MNDs in humans including Amyotrophic Lateral Sclerosis (ALS), atypical ALS and late-onset spinal muscular atrophy. In recent years, *Drosophila* has proven to be a very powerful and flexible model system for studying human neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's diseases. Despite the progress made in the previous pathologies, research on MNDs has been stalled somewhat due to the lack of a versatile genetic system.

Objectives: To understand the patho-physiology underlying VAP-induced MNDs in humans, we undertook a detailed functional characterization of VAP proteins in flies (1).

Methods: By using transgenic and loss-of-function approaches we generated a model of VAP-induced MNDs in *Drosophila*. Extensive phenotypic analysis was performed to dissect the patho-mechanism underlying these devastating diseases.

Results: We found that *Drosophila* VAP-33 (DVAP-33A), the structural homologue of hVAPB in flies, regulates synaptic remodelling by affecting the size and number of boutons at neuromuscular junctions (NMJs). Associated with these structural alterations, are compensatory changes in the physiology and ultrastructure of NMJs which maintain synaptic transmission within functional boundaries. Loss of DVAP-33A also induces axonal path-finding defects in Mushroom Bodies (MBs), the brain centres controlling learning and memory in flies. This phenotype can be rescued by targeting the expression of DVAP-33A in MBs. We also found that hVAPB and DVAP-33A are functionally interchangeable since the expression of hVAPB in neurons rescues the lethality, the morphological and the electrophysiological phenotypes associated with DVAP-33A loss-of-function mutations. Moreover, transgenic expression of hVAPB in neurons induces phenotypes similar to the overexpression of DVAP-33A. These data clearly indicate that the human and the *Drosophila* proteins perform homologous functions at the synapse. We also found that transgenic expression of DVAPP58S (the *Drosophila* protein carrying the pathogenic mutation) in neurons recapitulates several hallmarks of the human diseases, including locomotion defects, neuronal apoptosis and aggregate deposition. We are currently performing a modifier screen aimed at identifying VAP-interacting proteins and the relative data will be presented.

Conclusions: These findings point to a possible role of hVAPB in synaptic homeostasis and emphasize the relevance of our model in elucidating the patho-physiology underlying motor neuron degeneration in humans.

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C75 AN *SOD1* MISSENSE MUTATION IN DOGS WITH DEGENERATIVE MYELOPATHY: A SPONTANEOUS ANIMAL MODEL FOR AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: Canine, Degenerative Myelopathy, SOD1 mutation

Background: Canine degenerative myelopathy (DM) is an adult-onset neurodegenerative disease, characterized by progressive pelvic limb paresis and ataxia.(1) If euthanasia is delayed, the clinical signs will ascend causing flaccid tetraparesis/plegia. Axonal and myelin degeneration of the spinal cord is consistently most severe in the dorsal portion of the lateral funiculus within the mid-thoracic to lumbar region.(1,2) Although DM is most commonly diagnosed in German Shepherd Dogs, it occurs in many other breeds and has been reported most recently in the Pembroke Welsh Corgi.(2) Amyotrophic lateral sclerosis (ALS) is an adult-onset, progressive paralysis of humans characterized by loss of motor neurons and sclerosis of the lateral funiculus. Mutations in the superoxide dismutase 1 (*SOD1)* gene cause some forms of familial ALS.

Objectives: The purpose of this study was to identify the gene and mutation responsible for canine DM.

Methods: Genome-wide association mapping of DM was performed with 38 cases and 17 controls from the Pembroke Welsh Corgi breed using the Affymetrix Canine Genome 2.0 Array™.(3) The strongest association was detected on CFA31 ($p_{raw} = 1 \times 10^{-5}$,$p_{genome} = 0.18$) where all affected dogs were homozygous for a common haplotype from 28.91 Mb to 29.67 Mb (CanFam2.0) containing 3 genes: *SOD1*, *TIAM1* and *SFRS15*. Clinical similarities between DM and familial ALS made *SOD1* a viable candidate gene. Exons 2 to 5 of canine *SOD1* were resequenced from DM-affected and normal dogs.

Results: Resequencing of *SOD1* in normal and affected dogs revealed a G to A transition, resulting in an E40K missense mutation. Homozygosity for the A allele was associated with DM in five dog breeds (Boxer, Pembroke Welsh Corgi, German Shepherd Dog, Chesapeake Bay Retriever, and Rhodesian Ridgeback). To verify our localization of the DM mutation, we fine mapped 63 SNPs across a 1.9 Mb region in five breeds which segregate for DM. Affected dogs from all five breeds share a five SNP haplotype surrounding the E40K mutation and no other haplotype in the region was concordant with the recessive mode of inheritance and disease phenotype, providing strong evidence that the E40K mutation underlies the disease phenotype. Microscopic examination of spinal cords from affected dogs revealed myelin and axon loss affecting the lateral white matter and neuronal cytoplasmic

inclusions that bind anti-*SOD1* antibodies. Such inclusions are also a feature of some forms of ALS caused by *SOD1* mutations

Discussion and Conclusions: We identify DM as the first spontaneously occurring animal model for ALS. These dogs could be used to investigate the processes that underlie motor neuron degeneration in DM and ALS and to evaluate therapeutic interventions.

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SESSION 9B CLINICAL TRIALS & TRIAL DESIGN

C76 RESULTS OF A RANDOMIZED, CONTROLLED PHASE II TRIAL OF COENZYME Q10 (COQ10) FOR ALS

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Keywords: Coenzyme Q10, Clinical Trial

Background: CoQ10 is a mitochondrial co-factor and antioxidant. Mitochondrial dysfunction and oxidative stress are considered key pathogenic mechanisms in ALS.

Objective: To report the detailed results of the clinical trial of High Dose Coenzyme Q10 (CoQ10) in ALS (QALS study), an NIH-funded, two-stage, phase II, randomized, placebo-controlled, double-blind, multicenter clinical trial.

Methods: The QALS study had two aims: 1) to select between two doses of CoQ10 and 2) to conduct an early efficacy test of CoQ10 compared to placebo. The primary outcome measure was the decline in amyotrophic lateral sclerosis Functional Rating Scale-revised (ALSFRS-R) score from baseline to nine months. The first stage (dose selection) identified a preferred dose of CoQ10 (1800 mg or 2700 mg) using a selection procedure rather than a formal hypothesis test. The second stage (early efficacy test) compared this preferred dose against placebo using a non-superiority or futility design. A bias correction adjusted the early efficacy test result to take account of the inclusion of stage 1 data in the stage 2 analysis.

Results: A preferred dose (2700mg) was selected in December 2006 at the end of stage 1, using data from 70 patients (35 at each dose). Thirty five patients were concurrently randomized to the placebo in stage 1. In stage 2, an additional 80 patients were randomized 1:1 to CoQ10 2700mg and placebo. The stage 2 early efficacy analysis included 150 patients (75 on CoQ10 2700mg and 75 on placebo). The final stage 2 patients completed the trial in March 2008.

Discussion and Conclusions: A two-stage multicenter phase II randomized controlled trial of high-dose CoQ10 for ALS has been completed. The early efficacy test result for the primary outcome measure and limited safety data only was reported at the American Academy of Neurology Meeting in Chicago. For the International Symposium on ALS/MND, we will present the detailed efficacy and safety results and discuss our interpretation as to whether or not a definitive phase III trial comparing CoQ10 2700mg to placebo is justified.

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C77 SUBCUTANEOUS INSULIN-LIKE GROWTH FACTOR TYPE 1 (IGF-1) IS NOT BENEFICIAL FOR PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS IN A TWO YEAR TRIAL

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Keywords: Clinical trial; IGF-1; neurotrophic factors

Background: Previous human clinical trials of insulin-like growth factor, type I (IGF-1) in amyotrophic lateral sclerosis (ALS) have been inconsistent. One large study in North America suggested a beneficial effect as measured by the Appel ALS rating scale and one large European study failed to demonstrate benefit. We completed a multicentered, NIH funded study to assess the efficacy of subcutaneous IGF-1 in slowing the progression of ALS.

Objective: To determine if subcutaneously IGF-1 is effective at slowing the rate of progressive weakness in subjects with ALS. Secondary objectives: to determine if subcutaneous IGF prolongs survival or slows functional deterioration as measured by the ALSFRS-r.

Methods: This study was a randomized, double-blind, placebo-controlled study. 330 patients from 20 medical centers were randomized to receive 0.05 mg/kg body weight of human recombinant IGF-1 given subcutaneously twice daily or placebo for 2 years. The primary outcome measure was the rate of change in their manual muscle testing (MMT) score. Secondary outcome measures included tracheostomy-free survival and rate of change in the ALSFRS-r. Intention to treat analysis was used.

Results: There was no difference between treatment groups in the primary or secondary outcome measures after the 2 year

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treatment period. The IGF-1 treatment group MMT scores changed at a mean rate of 0.44 units per month and the placebo group changed at a rate of 0.39 units per month ($p = 0.716$). Survival analysis demonstrated a median survival of approximately 2 years with no difference between the treatment or placebo groups ($p = 0.415$). For the ALSFRS-r, the IGF-I treatment group changed at a rate of 2.5 units per month and the placebo group changed at a rate of 2.2 units per month ($p = 0.312$).

Conclusions: We found no evidence that subcutaneous IGF-1 benefits patients with ALS.

C78 TWO-YEAR PLACEBO-CONTROLLED RANDOMIZED TRIAL OF GENE THERAPY IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS.

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Keywords: gene therapy, VEGF, angigenin

Background: Polymorphisms in VEGF gene are associated with decreased expression of VEGF and increased risk of amyotrophic lateral sclerosis (ALS) (1). Mutations in angiogenin gene are detected in patients with ALS (2). Gene therapy with lentivirus vectors, expressing VEGF, in mouse G93A model increased survival time (3).

Objectives: We investigated safety, tolerability and effectiveness of gene therapy with recombinant adenovirus vectors, expressing VEGF and angiogenin, in ALS patients.

Methods: We conducted a two-year placebo-controlled randomized trial. 10 ALS patients with cervical onset of the disease were included in the study. They were randomized in two groups. Groups were comparable by age, sex and disease duration. We made intramuscular injections of medication in three muscles (m.trapezius, m.deltoideus, m.quadriceps) bilaterally every four weeks for two years. Every 4 weeks we conducted clinical and neurological examinations and laboratory tests. We also measured VEGF levels in serum and levels of adenovirus -neutralizing antibodies.

Results: All patients were deteriorating. There was no difference in the dynamics of forced vital capacity (FVC) and ALSFRS scores. In the group of patients receiving therapy, we registered increased survival under hypoxic conditions. We detected high level of virus neutralizing antibodies in one patient, receiving medication, who died during the first year of therapy. No serious adverse events were registered.

Discussion and Conclusions: We demonstrated safety and good tolerance of this therapy. We showed, that virus neutralizing antibodies may decrease effectiveness of therapy with recombinant adenovirus vectors. Gene therapy may increase resistance to hypoxia.

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C79 PYRIMETHAMINE AS A THERAPY FOR SOD1 ASSOCIATED FALS: EARLY FINDINGS

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Keywords: Pyrimethamine, familial ALS

Background: Three percent of ALS patients have a familial form of the disease (FALS) caused by a mutation in the gene coding for the free radical scavenging enzyme copper/zinc superoxide dismutase (SOD1). Inhibiting expression of the SOD1 gene prevents transgenic ALS animals from developing the disease. Increasing or decreasing the number of mutated genes proportionately speeds or slows the progression of the disease. Therefore, reducing SOD1 levels in patients with SOD1 associated FALS may be an objective for future therapies. Through an extensive in vitro screening program for medications having the ability to reduce SOD1 levels, several molecules that reduce SOD1 protein levels are known. One of the most potent molecules is pyrimethamine, an FDA approved medication used for the treatment of malaria and toxoplasmosis. Pyrimethamine reduces SOD1 levels in the spinal cord and blood lymphocytes in the G93A SOD1 transgenic mouse.

Objectives: To describe changes in SOD1 levels and muscle strength in FALS patients receiving pyrimethamine over an 18 week time period.

Methods: After obtaining FDA and IRB approval, 7 patients with El Escorial definite ALS associated with an SOD1 mutation were enrolled in an 18 week open label trial. The protocol defined pyrimethamine dose to be increased from 25mg to 100mg in the first 6 weeks, remaining at 100mg through to week 18. Lymphocyte SOD1 levels were measured at each visit. Disease severity was measured by Appel score (AALS), ALSFRS, and MQOL at weeks 6, 12 and 18. Leucovorin, 10 mg per day was given throughout the 18-week study.

Results: Four different SOD1 mutations were identified in seven patients: D90A (2: 1 man; 1 woman), A4V (2: 1 man; 1 woman), L144F (2 men), N65S (1 woman). Adverse effects of variable severity were encountered in all patients. Two patients (N65S and D90A) were unable to complete the study because of adverse effects. In non-A4V patients, SOD1 levels showed medication dependent reduction in SOD1 levels between 30–60 percent. Pyrimethamine administration had no effect on SOD1 levels in the 2 patients with the A4V mutation. In the 18 week period, AALS, ALSFRS, and MQOL scores showed no significant change in non-A4V patients. There was, however, significant deterioration in patients with A4V mutations during the study period.

Discussion and Conclusions: Pyrimethamine is capable of reducing levels of SOD1 in mouse lymphocytes, mouse spinal cord and persistently in human lymphocytes to up to 60% of normal. However, patients with the A4V mutation seem resistant the effects of pyrimethamine. While the safety and tolerability of PYR in FALS patients remains unsettled, we believe determination of an optimal dose will decrease adverse events. Larger trials to determine clinical efficacy and possible mutation specific effects are needed.

C80 A BAYESIAN MODEL TO DESIGN TWO STAGE CLINICAL TRIALS IN ALS

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Keywords: Trial design, historical controls, screening

Background: Clinical trials in ALS are generally designed one-at-a-time, testing a single drug. In reality, however, many drugs are waiting in a pipeline for testing and patients are eager to enter trials, especially if they can avoid being placed in a placebo group. We explore whether it is possible to design a series of trials, which can efficiently test several drugs using a consortium of clinical trial treatment centers.

Objective: To investigate, using a Bayesian model simulation, whether a two-stage sequential strategy can result in greater efficiency in finding useful drugs for treating ALS.

Methods: We simulated a process where there is a pool of drugs waiting to be tested for efficacy in ALS. We assumed that the effect of drugs on ALSFRS-R rate of decline (slope) comes from a normal distribution, with a specified mean. The standard deviation of the pool is used to specify the percentage of drugs that are truly effective, i.e. above a specified threshold. Testing occurs sequentially in two stages. First a drug is selected from the pool and in the initial "screening" stage patients taking the selected drug are compared to a pool of historical placebo control results. If the drug passes the screening test, a sample size is calculated, based on the estimated effect size from the first stage, for a second stage, randomized placebo controlled trial. If the drug does not pass the screen, another drug is selected for testing.

We investigated the effect of the theoretical distribution of drug efficacies on the optimal sample size for the first stage of the trial, based on total number of patients required to find effective drugs in the two-stage design.

Results: We modelled a scenario with 20% of drugs having a 20% or greater reduction in ALSFRS slope. The results are summarized in the text below where N is the number of patients selected for the screening stage:

The proportion of True Positive (a 20% or greater ALSFRS slope reduction) detected in a single-sample screening test when N was 30, 52%; 40, 61%; 50, 68% and 100, 88% respectively. The proportion of single-sample screening tests resulting in a False Positives when N was: 30, 31%; 40, 33%; 50, 32% and 100, 28% respectively. The average number of patients to test 10 drugs, screening plus a placebo-controlled phase III for 30 patients was 681, for 40 patients was 771, for 50 patients was 874 and for 100 patients was 983.

Conclusions: Small screening trials prior to large phase III trials can result in savings in numbers of patients required to find an effective drug.

C81 *TIME TO FAILURE* CLINICAL TRIAL FOR PROMISING THERAPEUTICS IN ALS: A NOVEL DESIGN FOR THE BEST OF BOTH WORLDS

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Keywords: clinical trial, study design, statistical methods

Background: The inclusion of a placebo or untreated control group in a randomized clinical trial testing novel therapeutics is necessary to control the placebo for the placebo effect in this heterogeneous disease and is required to detect a mild to moderate treatment effect. Successful recruitment requires a committed and selfless population of research subjects who may only have the opportunity of participating in one clinical trial. In addition, many subjects will never receive the active compound and will have a long exposure period to placebo or observation when mortality, or the rates of functional decline are the outcome measures.

To optimize recruitment, clinical trials designed for subjects with Cancer and HIV frequently employ a *time to failure* or *time to progression* design to minimize the duration of time research subjects receive the control treatment.

Objective: We evaluated a novel *time to failure* design for patients with ALS with time from randomization to a six unit drop in ALSFRS-R, death or permanent assisted breathing as the primary measure of treatment efficacy. Patients in the placebo group who decline by six units in the ALSFRS-R will be designated as treatment failures and switched to active treatment as soon as they reach this endpoint.

Methods: North Eastern ALS consortium's trials of creatine, topiramate, and celebrex were used to simulate how such a design would work in practice.

Conclusions: A total of 250 patients randomized 1:1 is required in a dual arm trial design to detect a 40% reduction in the rate of decline in the ALSFRS-R, with 90% power. A conventional trial design that used a random effect model to compare the rate of decline over 12 months would require 180 patients. However, the novel *time to failure* design would only expose patients to an average of seven months on placebo and patients with a rapid progression in the placebo group would receive active compound earlier in the course of their disease.

This novel trial design testing promising therapeutics in ALS provides a compromise between determination of efficacy and limiting the period of time patients are on placebo. This study design will be applied to an upcoming clinical trial of Lithium in the United States and Canada and details of study design along with advantages and disadvantages will be discussed.

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SESSION 10A SOD1 PATHOGENESIS

C82 THE POTENTIAL ROLE OF SOD IN THE PATHOGENESIS OF SPORADIC ALS

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More than 90% of ALS is of sporadic nature. Molecular mechanisms of sporadic ALS (sALS) are more difficult to study compared to those of known inherited forms of ALS. Studies of the SOD1-linked familial form of ALS over the last 15 years have developed increasing amounts of information and knowledge, together with a realization that the immense complexity of sALS limits the application of this model. Different approaches to identify genes and proteins that are involved in sALS will be reviewed. Recent work on a known blood enzyme that may be potentially important in sALS will be reported. Finally, thoughts on how one might approach studying sALS in the context of other neurodegenerative diseases that are largely of a sporadic nature will be discussed.

C83 MODULATION OF ENDOPLASMIC RETICULUM STRESS AS A POTENTIAL TREATMENT FOR ALS

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Keywords: SOD1, aggregation, ER stress

Background: The causes of motor neuron death in ALS remain unknown, although protein misfolding and accumulation of disulfide bonded mutant SOD1 aggregates is linked to neurotoxicity in some familial forms of disease. The unfolded protein response (UPR), a homeostatic mechanism activated by accumulation of misfolded proteins within the endoplasmic reticulum (ER), is activated in mutant SOD1 expressing transgenic rodents as well as in spinal cords of human patients with non-SOD1 linked sporadic ALS. The UPR involves upregulation of chaperones and down-regulation of general protein synthesis, however if the process is prolonged ER stress occurs and apoptosis is triggered via CHOP, JNK and ER stress-specific caspases. Protein disulfide isomerase (PDI), a key ER stress response chaperone with disulfide bond modulating activity, is also upregulated as part of the ER stress response and is protective against mutant SOD1 aggregation *in vitro*.

Objectives: The aim of this study was to examine the effects of a small molecular mimic of PDI and ER stress modulating drugs on mutant SOD1 aggregation and ER stress in ALS models.

Methods: Motor neuron-like NSC34 cell lines expressing either wildtype or mutant SOD1-EGFP fusion proteins were treated with a small molecular mimic of PDI or pharmacological modulators of ER stress. Immunocytochemistry, confocal microscopy and immunoblotting were performed to detect protein inclusions and high-molecular weight insoluble protein aggregates, as well as markers of ER stress.

Results: Treatment with the PDI mimic or ER stress inhibitor decreased: the formation of large mutant SOD1 inclusions, levels of insoluble SOD1 protein and toxicity. Conversely, pharmacological induction of the ER stress response had the opposite effect. Mutant SOD1 induced expression of ER stress response proteins, including the apoptotic transcription factor CHOP, this induction was decreased in cells treated with either the PDI mimic or ER stress inhibitor.

Discussion and Conclusions: We have shown that a small molecule mimic of PDI retains PDI-like activity in decreasing mutant SOD1 aggregation and preventing induction of ER stress, and that inhibition of ER stress also decreases protein aggregation *in vitro.* These data suggest that PDI and the ER stress pathways are possible therapeutic targets for treatment of ALS, and further *in vivo* experiments will clarify whether or not these compounds could be beneficial in altering ALS disease course.

C84 TRAFFICKING FROM ER TO GOLGI IS DISRUPTED IN MUTANT SOD1 EXPRESSING CELLS

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Keywords: ER stress, cellular trafficking, SOD1 aggregation

Background: We and others showed recently that (a) SOD1 is secreted by an ER to Golgi-dependent route and dysfunction of the secretory pathway is linked to neurotoxicity in ALS and (b) that Endoplasmic reticulum (ER) stress is present in lumbar spinal cords of transgenic $SOD1^{G93A}$ mice and human patients with sporadic disease. The up-regulation of ER stress specific apoptotic markers (caspase-12 and CHOP) in these tissues suggests that this is an important pathway to cell death in both forms of ALS.

Objective: To determine the relationship between secretory pathway dysfunction, SOD1 aggregation and ER stress in ALS.

Methods: NSC34 cells expressing mutant and wildtype SOD1 were examined by Western blotting and immunohistochemistry for the expression and distribution of ER and Golgi trafficking proteins.

Results: In cells expressing mutant SOD1, CHOP was upregulated prior to the formation of the aggresome, indicating that the smaller aggregates or oligomers are responsible for ER stress and toxicity in ALS. Trafficking proteins of the ER Golgi Intermediate Compartment (ERGIC) were downregulated in comparison to cells expressing wildtype SOD1. Vesicle associated protein COPII also had an altered distribution and co-localised with SOD1 aggregates. However, markers of the endosome pathway were not altered in these cells indicating that the post-Golgi secretory pathway was not affected by mutant SOD1 expression.

Conclusion: Small 'microaggregates' or oligomers of mutant SOD1 lead to ER stress, dysfunction of the ERGIC compartment, and neurotoxicity.

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C85 ZINC-DEFICIENT MONOMERS ARE WELL-POPULATED SOD1 UNFOLDING INTERMEDIATES: IMPLICATIONS FOR AMYOTROPHIC LATERAL SCLEROSIS PATHOGENESIS

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Keywords: SOD1, protein folding, kinetics

Background: Mutations in the Cu,Zn superoxide dismutase (SOD1) are known to cause a subset of cases of familial amyotrophic lateral sclerosis (ALS). This protein is a homodimer in which each monomer binds one catalytic copper atom and one structural zinc atom. Mutant SOD1 cytoxicity is believed to be due to increased conformational flexibility, which either allows aberrant substrates to access the SOD1 active site, giving rise to novel toxic catalytic activity, or increases the propensity of SOD1 to misfold and to aggregate. SOD1 protein stability is greatly affected by its metal ligands, but little is known about the role these ligands play in the folding, unfolding, and misfolding processes.

Objectives: This study aims to generate a more detailed model of the SOD1 unfolding process, focussing particularly on the release of bound copper and zinc as the protein denatures. Such a model would identify potentially disease-relevant folding intermediates and describe their metallation states. These intermediates may be useful targets for therapeutic agents designed to slow or halt ALS disease progression.

Method: SOD1 denaturation in molar concentrations of guanidine HCl was monitored in several ways. A variation of the 4-(2-pyridylazo)resorcinol (PAR) assay used previously to quantify SOD1 metal content was employed to measure rates of copper and zinc release. Rates of local conformational changes in the SOD1 beta-barrel were measured by changes in tryptophan fluorescence intensity. A time-resolved glutaraldehyde cross-linking assay was employed to quantify the rate of dimer dissociation.

Results: Distinct rates and mechanisms of copper and zinc release were observed. Where zinc is released rapidly, by a simple two-state mechanism, copper release was a slower process showing a distinct lag phase that could only be fit by a three-state sequential release model. Dissociation of the SOD1 dimer and conformational changes in the SOD1 beta-barrel monitored by tryptophan fluorescence occurred by two-state mechanisms.

Discussion and Conclusions: SOD1 unfolds by a complex mechanism involving a number of distinct processes, including release of bound copper and zinc, dissociation of the SOD1 homodimer, and conformational changes in the SOD1 beta-barrel. Correlations in the rates of these processes across a range of guanidine concentrations reveal linkages in them. Dimer dissociation and zinc release appear to happen rapidly and simultaneously. Copper release occurs over a much longer period of time, and is necessarily preceded by a conformational change in the SOD1 beta-barrel. The majority of SOD1 molecules denature by a four-state mechanism, in which zinc release and dimer dissociation occur first followed by the slower beta-barrel conformational shift, which in turn is followed by rapid copper release. This establishes a zinc-deficient, copper-loaded SOD1 monomer as a well-populated SOD1 unfolding intermediate, and a species likely to be populated under conditions of denaturational stress. Since this species has previously been reported to possess enhanced neurotoxicity in cell culture and enhanced aggregation propensity, this is a likely candidate to be the disease-causing neurotoxic species in SOD1-associated ALS.

C86 ISOLATION AND PROTEOMIC CHARACTERIZATION OF MUTANT SOD1-CONTAINING INCLUSION BODIES

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Keywords: SOD1, proteomics, inclusions

Introduction: A characteristic finding in ALS patients and transgenic murine models carrying SOD1 mutations are inclusion bodies displaying SOD1-immunoreactivity. A possible toxic property of mutant SOD1 is interaction with proteins that become inactivated, depleted or erroneously activated. These proteins could co-aggregate with SOD1 and become part of cellular inclusion bodies.

Objectives: To identify proteins in SOD1 inclusion bodies.

Methods: Spinal cord homogenates from terminal G127X and G85R mice were subjected to ultracentrifugation in density gradients. From these separations fractions containing SOD1-inclusions, free from other organelles, could be isolated. There were no inclusions in corresponding fractions of presymptomatic or control animals. Relevant fractions were further evaluated by atomic force microscopy, 2-dimensional gel analysis/MALDI-TOF and by LC-MS/MS.

Results: In density gradient centrifugations SOD1 inclusions entered the gradient but showed a large heterogeneity in density. A portion displayed a density higher than that of any organelle (mitochondria, lysosomes or peroxisomes) or markers for plasma membrane. Prolongation of centrifugation time from 1.5 to 4 hr did not change the distribution pattern. By addition of 0.5% of the detergent NP-40 to the homogenates, virtually all SOD-1 material in the gradient appeared at the bottom of the gradient, as expected for aggregated proteins. This suggests that the inclusions are not simple protein aggregates but more complex in structure. By atomic force microscopy, the inclusions were mainly round shaped with a large heterogeneity in size. In 2D gels from G85R and G127X mice about 20–30 protein spots could be seen, most overlapping between the models. In control animals only a few or no protein spots could be found in the corresponding fractions. SOD1 accounted for about 50% of the total amount of protein in the inclusions. About ten proteins have so far been identified, some previously known from immunohistochemistry to be constituents of SOD1-inclusion bodies.

Discussion: By this method we are able to find previously unknown SOD1-aggregation partners as well as other proteins that might be trapped in these inclusions. Knowledge of the identity of such proteins might be valuable for the development of understanding the toxic mechanism of mutant SOD1s and the pathogenesis of ALS.

C87 ALS2/ALSIN-DEFICIENT SOD1^{H46R} TRANSGENIC MICE EXHIBIT INCREASED ACCUMULATION OF INSOLUBLE PROTEINS IN THE SPINAL CORD

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Keywords: ALS2/alsin, SOD1, protein degradation

Background: *ALS2* is a causative gene for a juvenile autosomal recessive form of motor neuron diseases (MNDs), including ALS2, PLSJ, and IAHSP. The *ALS2*-coded protein, ALS2/alsin, activates Rab5 small GTPase and involves in macropinocytosis-associated endosome trafficking and fusion, and neurite outgrowth in the cells. Loss of these functions accounts for motor dysfunction and axonal degeneration in the *ALS2*-linked ALS/MNDs. Recently, we have shown that loss of ALS2 exacerbates motor dysfunction in SOD1^{H46R} mice, indicative of a close relationship between the ALS2-associated cellular function and mutant SOD1-mediated pathogenesis *in vivo*. However, molecular mechanisms for the acceleration of disease progression in ALS2-deficient/mutant SOD1-expressing mice are still unknown.

Objectives: To clarify the biochemical basis of the ALS2-mediated functions that are associated with disease progression in SOD1^{H46R} transgenic mice.

Methods: We generated mice with 6 different genotypes; *Als2*$^{+/+}$ (wild-type), *Als2*$^{+/-}$, *Als2*$^{-/-}$, SOD1^{H46R}, *Als2*$^{+/-}$;SOD1^{H46R}, and *Als2*$^{-/-}$;SOD1^{H46R}, by crossing male *Als2*$^{+/-}$;SOD1^{H46R} and female *Als2*$^{+/-}$ mice. Brain and spinal cord tissues were obtained from these mice at 8, 12, 16 and 20 weeks of age. The Triton X-soluble and insoluble fractions were prepared and aliquots of samples were separated by SDS-PAGE. The various protein bands with an increased intensity as the disease progressed were excised and analyzed by liquid chromatography-tandem mass spectrometry with computational peptide mass fingerprinting (LC-MS/MS-PMF). Further, tissue samples were subjected to Western blot analyses. A panel of antibodies, which included anti-ALS2, anti-SOD1, anti-ubiquitin, anti-p62/SQSTM1, anti-GFAP, anti-vimentin, anti-neurofilament HC, anti-peripherin, anti-beta-tubulin, and anti-GAPDH antibodies, were used in this study.

Results: SOD1^{H46R}, *Als2*$^{+/-}$;SOD1^{H46R}, and *Als2*$^{-/-}$;SOD1^{H46R} mice all exhibited progressive motor dysfunction and paralysis. Notably, a mean survival of *Als2*$^{-/-}$;SOD1^{H46R} mice (152.4 ± 5.0 days; $n = 67$) was significantly shorter than those in SOD1^{H46R} (164.5 ± 11.1 days; $n = 146$) or *Als2*$^{+/-}$;SOD1^{H46R} mice (164.8 ± 10.3; $n = 190$) ($p < 0.001$). PAGE analysis of insoluble spinal cord proteins demonstrated two bands (p55 and p50) with an increased intensity as the disease progressed. LC-MS/MS-PMF analysis identified that p55 and p50 represent vimentin and GFAP, respectively. Although levels of neuronal intermediate filament proteins; neurofilament HC and peripherin were unchanged, other insoluble proteins such as vimentin, p62/SQSTM1, and mutant SOD1 were more progressively accumulated in the spinal cord of *Als2*$^{-/-}$;SOD1^{H46R} mice, from an approximately 8 weeks prior to disease-onset, than in those of SOD1^{H46R} or *Als2*$^{+/-}$;SOD1^{H46R} mice.

Discussion and Conclusions: Our results suggest that loss of ALS2 hinders protein degradation and/or accelerates the accumulation of a number of insoluble proteins such as astrocyte-associated intermediate filaments and autophagy-associated protein (p62/SQSTM1) in the spinal cord, thereby exacerbating motor dysfunction in SOD1^{H46R} mice. Further characterization of these mice will clarify the implication of the ALS2-mediated functions in mutant SOD1-linked ALS/MND *in vivo*.

C88 ANALYSIS OF THE ROLE OF DYNEIN MUTATIONS IN ATTENUATING THE PHENOTYPE OF SOD1^{G93A} TRANSGENIC MICE

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Keywords: dynein, Loa, SOD1

Background: Cu/Zn SOD1^{G93A} transgenic mice develop an ALS-like phenotype, which is characterized by motor neuron degeneration and muscle paralysis. Previously, we demonstrated that a missense point mutation in the gene encoding the heavy chain subunit of cytoplasmic dynein causes degeneration of motor neurons in the Legs at odd angles (*Loa*) mouse (1). We also showed that double mutant (*Loa*/SOD1^{G93A}) transgenic mice have a significant improvement in SOD1^{G93A} -mediated disease, including a reduction in motor neuron degeneration (2).

Here we present data indicating a subcellular redistribution of mutant SOD1 in the *Loa*/SOD1^{G93A}, which could provide a mechanistic explanation for the amelioration of the disease in these mice.

Objectives: To elucidate at the molecular level the role of the dynein mutations in attenuating the phenotype of SOD1^{G93A} transgenic mice.

Methods: We have used density and buoyant gradient sedimentation assays to fractionate homogenates from the mouse spinal cord and brain tissues into subcellular fractions. Fractions were analysed on SDS-PAGE and Western blots.

Results: Our density gradient sedimentations of SOD1^{G93A} protein in tissues from SOD1^{G93A} and *Loa*/SOD1^{G93A} littermates show a clear difference in the sedimentation patterns of this protein in the brain and spinal cord tissues from these mice. There is significantly more SOD1^{G93A} protein in the denser fractions of the 90 and 121-day SOD1^{G93A} mice compared with those of *Loa*/SOD1^{G93A}. Importantly, these denser fractions contain mitochondria, as determined by using an antibody to the mitochondrial marker COX4.

Discussion: Our previously reported amelioration of the disease phenotype in SOD1^{G93A} by defective dynein highlighted the dynein mediated retrograde axonal transport as a potential target for therapy. The dramatic depletion of SOD1^{G93A} from denser cellular fractions, containing mitochondria, of *Loa*/SOD1^{G93A} in density gradient sedimentation assays suggests that mutant dynein alters the sub-cellular distribution of toxic SOD1^{G93A} protein, which may reflect a

decreased level of association of mutant SOD1 with mitochondria. In support of this hypothesis we have evidence that the mitochondrial membrane potential is depolarized in $SOD1^{G93A}$ spinal cord, but in *Loa*/$SOD1^{G93A}$ it returns to normal levels (abstract submitted separately by V. Bros). We are currently investigating the mitochondrial function in $SOD1^{G93A}$ versus *Loa*/$SOD1^{G93A}$.

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SESSION 10B RESPIRATORY MANAGEMENT

C89 THE ALS NUTRITION/NIPPV STUDY

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Keywords: Nutrition, NIPPV, early intervention

Background: Intervention with nutritional support and NIPPV are important in the management of ALS and are thought to prolong survival and maintain quality of life. The optimal timing of these interventions has yet to be defined. Currently, nutritional support via PEG is advocated based on symptoms (e.g., dysphagia, weight loss) and NIPPV on the basis of FVC. Ideally, recommendations for these interventions should be based on evidence of either nutritional or ventilatory insufficiency.

Objectives: The objectives are: 1) to develop equations to predict total daily energy (calorie) expenditure (TDEE), 2) to evaluate acceptance, compliance, and tolerability of NIPPV at FVC 50% vs 80%, 3) to examine early predictors of respiratory insufficiency, and 4) to evaluate the effect of interventions on QOL of patients and caregivers.

Methods: In a multi-center pilot study, ALS patients with FVC > 50% of predicted were entered into a nutrition arm (n = 80) or a NIPPV arm (n = 73) and studied at baseline, 16, 32 and 48 weeks. In the nutrition arm, TDEE was measured using the "gold standard" doubly labelled water (DLW) method to serve as the reference against which predictive equations will be developed. Other factors measured longitudinally include: demographics, ALSFRS, FVC, body composition (BIS, DXA), physical activity (accelerometers, Bouchard scale), resting metabolic rate, clinimetric scales (spasticity, cramps, fasciculations, fatigue), food intake, laboratory measures, and psychosocial scales. Subjects in the NIPPV arm were divided into an Early Intervention Group (Group 1; NIPPV initiated with FVC 75–85%) and the Standard of Care Group (Group 2; NIPPV initiated with FVC 45–55%). Acceptance, tolerability, and compliance were determined by self-report and NIPPV meter readings. Longitudinal measures of ventilatory status were determined (SNP, NIV, MVV, supine FVC, scales) to evaluate early changes.

Results: 153 ALS patients were enrolled from 12 participating study sites (5, nutrition arm; 7, NIPPV arm). The characteristics of the subjects at baseline were: age (54.9 ± 11.5 yrs), gender (M/F; n/%; 98/64%; 55/36%), site of onset (limb/bulbar, n/%; 115/75%, 38/25%), months from onset to enrolment (23.5 ± 18.1), and ALSFRS (35.6 ± 6.2). Entry characteristics were comparable in the two study arms. For analysis and modelling, the nutrition participants were divided into 4 groups based on their entry FVC (>80% vs 50–80%) and on ambulatory status (ALSFRS question 8; "4" vs "3, 2, 1, or 0"). Although analyses for TDEE are in progress, measurements to date indicate TDEE ranged between 1199 and 3614 kcal/d with a mean of 2276 ± 600 kcal/d at baseline. Longitudinal assessments of TDEE, water intake, calorie intake and physical activity are in progress to complete modelling procedures. 55 out of 73 NIPPV participants reached criteria for intervention with NIPPV.

Discussion and Conclusions: We project that we will have >200 individual TDEE determinations and associated clinical measures to successfully develop ALS-specific predictive equations for TDEE as the disease progresses. Statistical analyses are in progress to evaluate factors contributing to NIPPV acceptance and tolerance to project sample size for a definitive trial of early NIPPV intervention.

C90 PREDICTIVE VALUE OF RESPIRATORY TESTS FOR RESPIRATORY INSUFFICIENCY IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: Respiratory function tests, hypercapnia, phrenic nerve stimulation

Background: A large number of respiratory function tests (RFT) have been studied to detect earlier signs of hypoventilation in amyotrophic lateral sclerosis (ALS). However, few reports have studied the predictive values of those tests for global hypoventilation. This is important as non-invasive ventilation (NIV) can correct hypoventilation.

Objectives: We investigated the predictive value of a large number of volitional and non-volitional respiratory tests for hypoventilation in ALS. In particular, we aimed to address phrenic nerve motor response.

Methods: We analyzed data from 199 ALS patients with probable or definite disease (revised El Escorial criteria) followed in our Unit. Sixty-eight patients had bulbar-onset (G1) and 131 spinal-onset (G2). Twenty-five had hypercapnia (pCO_2 >45 mmHg), which was considered the "gold standard" to define hypoventilation. The following measurements were recorded: forced vital capacity (FVC); maximal static inspiratory and expiratory pressures (PImax and PEmax); mouth inspiratory pressure at 100ms (P0.1); amplitude of the motor responses to phrenic nerve stimulation (Ampl). We analyzed the results obtained at these patient's first visit to our Clinic. For each group the sensitivity and specificity of positive (PPV) and negative (NPV) predicted values, as well as their accuracy as determined by ROC (receiver operator characteristics) analysis were determined. A logistic regression model was applied to identify test results that were significantly related to hypercapnia.

Results: The mean age was 61 years and 99 (50%) patients were women. The mean disease duration was 17.2 months. The disease onset was bulbar in 68 patients and spinal in 131 (in upper limbs in 63). Twenty-five (12.6%) patients had hypercapnia at the first observation. Defining the "cut-off" point by the normative values, PPV was low and NPV high for all measurements. ROC analysis and a logistic regression model confirmed that FVC (cut-off = 63.4%) and Ampl (cut-off = 0.25mV) was discriminative for the whole group of 199 patients, and G1, and Ampl (cut-off = 0.37mV) for G2.

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Discussion: Phrenic nerve stimulation is a simple, comfortable and objective test that seems useful to predict hypoventilation in ALS, in both spinal and bulbar-onset patients. FVC was predictive for bulbar patients but not for spinal-onset patients. This might be because decreased expiratory efficacy influenced the value of FVC as a surrogate for diaphragm function. We assessed central respiratory drive by measuring P01; we concluded that central hypoventilation does not seem relevant in bulbar-onset patients. Maximal inspiratory pressure is sensitive in detecting early respiratory change but does not predict global hypoventilation.

C91 FACTORS ASSOCIATED WITH NON-INVASIVE POSITIVE PRESSURE VENTILATION (NIPPV) COMPLIANCE IN PATIENTS WITH ALS/MND

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Keywords: Non-invasive positive pressure ventilation, respiratory management, compliance

Background: Despite growing evidence demonstrating that non-invasive positive pressure ventilation (NIPPV) prolongs survival and improves quality of life, a large percentage of patients initiated on this treatment remain non-compliant. Prior reports have suggested that compliance is most strongly correlated with symptoms of orthopnea and dyspnea (1,2).

Objectives: To prospectively examine factors which influence NIPPV compliance in patients with ALS/MND.

Methods: Seventy-three ALS patients with a forced vital capacity (FVC) >50% were enrolled in the multi-center pilot study of Early Nutrition and NIPPV. Patients with a FVC over 80% at baseline were initiated on NIPPV when their FVC was 75–85% (Group 1 – Early intervention). Patients with a FVC between 50–80% were started when their FVC was 45–55% (Group 2 – Standard of Care). Patients received an educational video prior to the initiation of NIPPV and the information presented was discussed in detail with the site investigator. A respiratory therapist made home visits three times a week for the first week, twice the second week, once the third and fourth weeks and then monthly throughout the study period. NIPPV compliance was defined as consistent use of at least >4 hours per night based on machine meter readings and patient report. Chi-square and t-test were used to determine the association between compliance and participants' characteristics at the time of NIPPV initiation.

Results: Of the 55 patients that initiated NIPPV, 35 patients (64%) were compliant with NIPPV. NIPPV compliance was associated with income over $50,000 ($p = 0.004$), higher educational level ($p = 0.04$). Compliance rates were 51% in patients initiated on NIPPV with an FVC between 75–85% and 82% in patients initiated on NIPPV with an FVC between 45–55% ($p = 0.02$). Age, gender, and site of disease onset did not predict compliance. There were no differences in compliance based on symptoms of dyspnea and orthopnea as measured by the Medical Research Council Modified Dyspnea Scale (MDS), the Borg Dyspnea scale, or the ALS Functional Dyspnea Score (ALSFDS), nor with the ALSFRS-R total score, upper extremity function, respiratory, or bulbar domains.

Discussion and Conclusions: This data suggests that the most predictive factors of NPPV compliance are advanced education and income >$50,000. The high overall compliance rate in this patient sample suggests the importance of patient education regarding the use of NIPPV as well as the need for aggressive respiratory therapy intervention at the initiation of treatment. Early initiation of NIPPV does not appear to improve tolerance.

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C92 WIRELESS PATIENT MONITORING FOR THE CONTROL OF COMPLIANCE TO NIV IN ALS PATIENTS: FUNCTIONAL AND SURVIVAL OUTCOME

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Keywords: Non-invasive ventilation, compliance, breathing pattern

Background: Non-Invasive Ventilation (NIV) is an efficient method to treat respiratory insufficiency in ALS as it improves survival and quality of life in compliant patients. Non-compliant patients are recognized when daily ventilator usage is < 4 h/d. Recent technological advances allow the identification of abnormal breathing patterns which is frequently the cause of intolerance leading to therapy abandonment. Real time actions based on this evidence may facilitate decision-making and reduce the process of trial and error and costs.

Objectives: To evaluate the impact on function and survival of ALS ventilated patients, whose ventilator settings are controlled via modem communications, whenever a detection algorithm is perceived, from the received data, an abnormal breathing pattern the patient's medical attendant is called.

Methods: 54 probable or definite ALS, consecutive and ventilated patients (17 bulbar-onset and 37 spinal-onset) in the last three years, were randomized according to the locality of residence to check compliance and parameters settings in office visits (G1:n = 28) or to receive a device for remote controlling the Goodknight 425 ST (G2:n = 26). This machine forwards weekly data to an e-health monitoring room. All patients were followed-up every three months with RFT, ALS-FRS. Throughout the clinical evolution, we registered the number of office visits, the number and type of each parameters changed, breath frequencies, the percentages of spontaneous breathing, of attained predetermined pressures, of the total ventilator usage in days, of days with use greater than 6 hours. We also recorded survival from symptom onset and from NIV adaptation. Groups were compared and data analysed to find differences at admission in order to control for the confounding variables and subsequently identify possible predictors of long-term satisfaction (compliance), survival and functional outcome.

Results: G1 (9 bulbar-onset and 19 spinal-onset mean ages ± SD 61 ± 1,6), G2 (8 bulbar-onset and 18 spinal onset with mean ages ± SD 59 ± 2). There were no differences at admission regarding age, gender, type of disease-onset and disease duration. The number of non-compliant patients was lower in G2 though not statistically significant. The number of changes in the parameter settings was not different although the number of days to full compliance was significantly lower in G2. Total survival from symptoms onset until January 2008; as well as the total amount of ventilator usage in days was significantly increased in G2. There were significant differences regarding initial parameter settings, showing lower inspiratory pressures (IPAP), breathing frequencies in G2 and lower percentages of spontaneous breathing in G1.

Conclusions: This study shows that remote control is able to reduce the trial and error procedures, speeds up the process of NIV adaptation and therefore may contribute to the increased survival observed.

C93 ELECTIVE TERMINATION OF NON-INVASIVE AND MECHANICAL VENTILATION IN ALS

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Keywords: termination of ventilation, discontinuation of therapy, palliative care

Background: Due to the growing use of ventilation therapy in ALS, physicians are increasingly confronted with patients who seek a discontinuation of non-invasive or mechanical ventilation. In Germany, termination of ventilation is legal and medico-ethically settled, yet there are few systematic investigations into withdrawal of ventilation therapy.

Objectives and Methods: In a retrospective investigation of 9 German ALS patients, clinical data were recorded from the discontinuation of non-invasive ventilation (n = 4) and mechanical ventilation (n = 5).

Results*:* In cases of residual spontaneous breathing (non-invasive ventilation, n = 3), an intensified symptom control of dyspnea and anxiety was possible with intravenous morphine sulfate (MSI) in a low dose rate (10 mg/h) but high cumulative dose (185–380 mg). The terminal phase after removal of the mask was protracted (22:10 h to 28:00 h). In cases of minimal or absent spontaneous breathing (invasive ventilation, n = 5; continuous non-invasive ventilation, n = 1), the disconnection was realized in a deep sedation, which required a moderate total dose (120 mg) but a high dosage rate (up to 300mg/h) of MSI. The terminal phase in deep sedation was short (15–80 min). In 2 patients, there were hypoxic myoclonus and automatisms.

Conclusion: The elective termination of ventilation requires a differentiated pharmacologic palliative care. In the future, controlled studies are required in order to establish evidence-based guidelines for the termination of ventilation.

C94 RESULTS OF PROSPECTIVE PILOT AND MULTI-CENTER PIVOTAL TRIALS OF DIAPHRAGM PACING IN AMYOTROPHIC LATERAL SCLEROSIS: MAINTAINING DIAPHRAGM FUNCTION AND IMPROVING SURVIVAL

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Keywords: diaphragm pacing, respiratory control, breathing

Background: Respiratory insufficiency is the major cause of mortality in patients with ALS/MND. Present therapies in ALS do not address diaphragm dysfunction. The diaphragm pacing system (DPS) is a standardized minimally invasive laparoscopic technique that is being utilized worldwide to maintain and provide natural diaphragm ventilation.

Objective: To analyze the safety and efficacy of DPS in ALS.

Methods: At 11 worldwide sites, a prospective lead-in design study obtaining a battery of three initial data sets were collected prior to treatment by laparoscopic placement of intramuscular diaphragm pacing electrodes. Subjects utilized DPS to condition their diaphragm with additional utilization depending on respiratory condition. Data was collected for another nine months post implant and until death.

Results: Recruitment was completed with 140 subjects (20 in pilot and 120 in pivotal with 20 European subjects). Of these 140 patients, 73 have been implanted, with 40 patients' still in the lead-in and 27 patients falling out after consenting but prior to implantation (most commonly from FVC not meeting inclusion criteria- 13 patients). Patient demographics at implantation were 70% male, 60% NIPPV use, 34% use of gastrostomy, FVC 60 ± 12% predicted with decline rate of 1.84% per month and ALSFRS-r score of 28 ± 7.5. There were no perioperative mortalities with over 20% having FVC below 50 and over 30% having elevated pCO_2. Non-device peri-operative morbidity included: one extended hospitalization from a pre-existing cardiomyopathy and one post-operative gastrostomy tube dislodgement requiring re-hospitalization. Total cumulative use of DPS is over 600 months with longest implants over 2 years with no non-tolerance of pacing post implantation. Subjects with declining FVC during the lead-in period showed a significant ($p < 0.05$)

improvement in paired comparison to rate of decline with treatment with an average improvement of $1.33 \pm 2.45\%$ per month. Overall there have been 19 patients that have reached the endpoint of death or tracheostomy with only 4 being the result of respiratory failure. The probability of survival is at a mean of 60.9 ± 6.7 months for all patients. In patients undergoing DPS and gastrostomy ($n = 18$) the 30 day survival was 100% and the 1 year survival 75%. The ALSFRS-R score showed that while the overall symptoms of ALS progressed the respiratory subscore did not decline. Prior to implant the respiratory subscore represented 32% of the total and following treatment it was 41%. In paired comparison of pre- and post-treatment, the respiratory subscore increased by $8 \pm 12\%$ of the total ALSFRS-R score ($p < 0.001$). DPS can continuously assess diaphragm EMG activity which has allowed the identification of instability of respiratory control and central hypoventilation. Sleep assessments performed at one site showed 23 of 47 patients utilize DPS during sleep. Greater fluoroscopic diaphragm movement is visualized with DPS stimulation in patients with more upper motor neuron diaphragm involvement. DPS has been able to lower pCO_2 levels with increased daily use.

Conclusion: The DPS system can be safely implanted and utilized in ALS patients with a positive effect on diaphragm function. DPS minimizes the impact of NIPPV on diaphragm dysfunction and positively impacts patients with identified instability of respiratory control.

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SESSION 11 JOINT CLOSING SESSION

C95 STEM CELLS AND ALS/MND: WHERE ARE WE NOW?

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Keywords: stem cells, cell therapy, MND model

The hope of one-day producing new motor neurons to replace those lost in ALS/MND remains the holy grail of stem cell research for motor neuron diseases. However, connecting new upper or lower motor neurons to their targets remains an enormous challenge, which will require a significant amount of new research in cellular and animal models.

But there is far more going on in the stem cell field than simply motor neuron replacement. Astrocytes, the support cells of the brain and spinal cord, may also be affected in ALS/MND and it is now possible to grow human stem cells that can make astrocytes following transplantation. Furthermore, they can be modified to produce powerful growth factors such as GDNF and IGF-1 which, while potentially important for protecting motor neurons, are difficult to get into the brain and spinal cord any other way. Thus, combining stem cell replacement of astrocytes with growth factor therapy is far more practical than motor neuron replacement at the present time, and may be translatable to the clinic in the near future.

In addition to stem cells providing a potential new cell therapy for ALS/MND they may also provide a tool for studying the mechanisms underlying motor neuron degeneration. New studies have shown that induced pluripotent stem (iPS) cells can be generated from adult human skin samples. These iPS cells are identical in many respects to human embryonic stem cells and can be made to differentiate into motor neurons under the correct conditions. We and others have begun to generate iPS cells from patients with ALS/MND and spinal muscular atrophy (SMA). Motor neurons and astrocytes generated from these cells give a new source of important human tissue to study how the disease may lead to cell death. They may also be used to screen potential new drugs.

Clearly this is a very exciting time in the stem cell world – both from a cell therapy and drug discovery aspect. But hope and hype must be carefully balanced and new discoveries and therapies will take require significant time and effort over the next few years.

C96 IMMUNISATION APPROACHES TO THERAPY FOR ALS

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Keywords: SOD1, immunization

The finding that mutant SOD1 can be secreted and that extracellular SOD1 mutants can trigger the death of motor neurons in culture led us to test immunization protocols aiming to reduce the burden of SOD1 mutant in nervous tissue of mice models of ALS. Initially, vaccination approaches were tested in G37R *SOD1* mice using bacterially-purified recombinant SOD1 mutant protein as immunogen. We showed that vaccination of G37R *SOD1* mice by injections of adjuvant-SOD1G93A was effective in delaying disease onset and extending life span by over 4 weeks. However, we believe that passive immunisation approaches would be safer in future human ALS clinical tests. In order to develop a passive immunisation approach, we have derived mouse monoclonal antibodies against SOD1 species using standard hybridoma technology. This was done with the use of recombinant G93A SOD1 apo-form as immunogen. The apo-form of the protein was selected because it has been reported that in SOD1 lacking metal some regions of the molecule become more exposed to external environment, especially the hydrophobic β-strands. The concept is that regions normally buried in the native dimer would become exposed, thereby acting as antigens. Using this approach, we succeeded in generating 12 antibodies that bind specifically to SOD1 mutants but not intact WT SOD1. These conformation-specific antibodies can be used to detect the accumulation and distribution of misfolded human SOD1 species during disease progression in mutant SOD1 mice. Misfolded SOD1 species are first detected in motor neurons and then spread out to other cell types as the disease progresses. Using immunoprecipitation (IP) assays, we found that some of these monoclonal antibodies can detect specifically misfolded SOD1 species in spinal cord extracts of fALS with $SOD1^{A4V}$ mutation. Moreover, our antibodies were able to detect misfolded SOD1 species in autopsy spinal cord samples from sporadic ALS patients using IP assay or immunohistochemistry examination. These data support the view that SOD1 misfolding might represent a common pathogenic pathway to fALS and sALS. We have tested a passive immunization approach through intraventricular infusion of conformation specific antibodies using osmotic minipumps starting at 90 days of age in G93A *SOD1* mice. Such immunotherapy succeeded in prolonging the lifespan of $SOD1^{G93A}$ mice. For development of human ALS immunotherapy, strategies are now being considered to derive humanised antibodies.

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THEME 1 THERAPEUTIC STRATEGIES

P01 NEW APPROACHES TO THE ANALYSIS OF OBSERVATIONAL DATA ON THE USE OF RILUZOLE IN ALS

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Keywords: Riluzole, effectiveness, analysis

Background: Although further study of the efficacy of riluzole was suggested by NICE in 2001 all new data since then has come from observational studies rather than RCT's.

Objective: To apply more rigorous methodology for analysing observational data through auditing the use of riluzole in a large MND Centre.

Methods: Data on survival from first symptom were recorded in 548 ALS patients. Date of diagnosis was available in 450 of these along with data on gender, onset site and onset age. We used Kaplan-Meier (KM) analyses and log-rank tests to assess categorical variables (including treatment) for univariable association with survival, and univariable Cox regression to assess significance of continuous variables. Variables with a univariable p-value of ≤ 0.2 were considered for inclusion in the multivariable Cox regression model. Riluzole and PEG treatment were fitted as time varying covariates. We examined survival from onset and diagnosis as two separate outcomes.

Results: Univariable KM analyses showed that onset site, riluzole and PEG status were significant predictors of survival from onset. Multivariable Cox regression showed that patients on riluzole had a 67% increased hazard of death (HR 1.67, 95%CI: 1.38–2.02) compared to patients who had not taken riluzole.

For analysis of survival from diagnosis, we only included patients who started riluzole within 95 days of diagnosis. In this model, only PEG was fitted as a time-varying covariate and age at diagnosis was used in place of age at onset. Multivariable Cox regression showed that age at diagnosis ($p = 0.001$), riluzole group ($p = 0.018$) and PEG group ($p < 0.001$) were significant predictors of survival from diagnosis. This model showed an advantage for riluzole (HR 0.78, 95%CI: 0.64–0.96). A patient receiving PEG has more than three times the hazard of death (HR 3.40, 95%CI: 2.71–4.27) compared to a patient not receiving PEG at that time.

Discussion: When analysing survival from onset using riluzole as a time-dependent covariate, only that proportion of the patient's journey while actually on riluzole was attributed to the treatment. The remainder of the patient's survival experience contributed to the non-treatment effect. The apparent disadvantage for riluzole in this analysis arose because of the time between first symptom and starting riluzole. Patients on riluzole were inevitably more advanced in the disease journey and thus at greater risk of death.

Patients generally start riluzole soon after diagnosis. The analysis based on survival from diagnosis showed a survival advantage broadly consistent with RCT data. We suggest that survival from diagnosis gives a more balanced comparison than survival from first symptom in interpreting the results of observational studies of riluzole.

P02 A PATIENT-LED TRIAL OF LITHIUM IN ALS USING THE INTERNET

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Keywords: Lithium, internet, trial

Background: In November 2007, a patient relayed an Italian news report about a promising result of a human trial to an online ALS community, PatientsLikeMe. The report described the apparent slowing of symptoms of ALS in patients taking lithium carbonate. In advance of formal publication of the study, a number of patients were prescribed lithium by their doctors off-label. A group of these patients began completing self-report ALSFRS-R scales and combining their data in a shared, online spreadsheet. To eliminate duplicate effort and to encourage standardised data collection, PatientsLikeMe enhanced its treatment recording and community report tools, with the result that over 160 ALS patients are tracking their use of lithium and reporting on disease progress and side-effects. This is alongside the remainder of over 1,500 ALS PatientsLikeMe users who are not using lithium, creating a unique opportunity to study the impact of lithium on a patient community.

Objectives: To study the evolution of a patient-lead open-label trial and examine how estimated effect sizes and other data in a non-experimental setting might inform the design of formal trials.

Methods: Patients who had obtained lithium carbonate through their own doctors were invited to enter self-report ALSFRS-R data on a regular basis, along with their lithium blood levels and any side effects they experienced. Participants were provided with a graphing tool that allowed them to compare, at a glance, the progression of all other patients taking lithium. Optional filters allowed users to customise the data displayed on the graph. All available data are being processed to develop statistical models of (a) the decision to use lithium, (b) the impact of lithium on ALS patients.

Results: At the time of writing, 162 patients with ALS were tracking their use of lithium, 10 times larger than the group reported in the Italian study. Before commencing lithium, the average rate of onset was 0.57 (S.D. 0.05) ALSFRS-R points per month, indicating a relatively slowly progressive sample relative to the known literature. Contrary to the Italian study, several users in our sample have died since starting lithium, and side effects have been reported including perceived worsening of respiratory function. Results for any detectable drug effects will be presented at the Symposium.

Discussion: The high level of internet use by ALS patients, the rapid flow of scientific information, and the ability to prescribe drugs off-label for compassionate use means that many patients will not wait patiently in line for a placebo-controlled trial. With sufficient improvements to data quality and safety monitoring, the use of a site such as PatientsLikeMe for a distributed clinical trial in ALS could prove time-efficient and cost effective.

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P03 GRANULOCYTE COLONY-STIMULATING FACTOR ADMINISTRATION IN AMYOTROPHIC LATERAL SCLEROSIS: A PHASE I/IIA, DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED CLINICAL TRIAL

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Keywords: granulocyte colony-stimulating factor, GCSF, clinical trial

Background: Recombinant human granulocyte colony-stimulating factor-GCSF (Neupogen®) is an approved drug used to mobilize CD34+ hematopoietic stem cells from the bone marrow to the peripheral blood. In previous studies it was shown that mobilized cells are able to cross the blood-brain barrier and can be found after treatment in brain tissue.

Objective: We hypothesized that autologous peripheral blood progenitor cells, mobilized from the bone marrow with GCSF, would slow down disease progression in patients with amyotrophic lateral sclerosis (ALS).

Methods: Patients with clinically definite or probable ALS of less than six years duration, with a forced vital capacity of at least 50% of predicted and limitation of motor function in at least one limb were randomly assigned in a double-blind fashion to receive either GCSF or placebo. GCSF was injected subcutaneously at a dose of 5μg/Kg once a day, for four consecutive days. Each treatment cycle was repeated every three months for a total of four cycles. The primary outcome measure was the decline over time of the revised ALS Functional Rating Scale score (ALSFRS-R). Secondary outcome measures were forced vital capacity, a manual muscle strength megascore derived from all limbs, compound muscle action potential amplitudes, neurophysiological index, a visual analogue scale for quality of life and tracheostomy-free survival.

Results: 39 patients (25 males) were enrolled, of them 19 received the active drug. 17 patients completed the one-year trial. The decline over time of ALSFRS-R, as well as of the secondary outcome measures, was not significantly different between placebo and active treatment groups. GCSF treatment did not alter survival of ALS patients in this study.

Conclusion: The presented treatment protocol had no beneficial effect on disease progression in patients with ALS.

P04 RECOMBINANT HUMAN ERYTHROPOIETIN THERAPY IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Keywords: Erythropoietin, intravenous, ALS functional rating scale-revised

Background: Amyotrophic lateral sclerosis (ALS) is a rapidly progressive, fatal neurodegenerative condition characterized by loss of upper and lower motor neurons in the brain and spinal cord. In spite of numerous researches, riluzole remains has been shown to have neuroprotective effects the only effective therapy, but with only minimal effects on survival. In previous reports, erythropoietin (EPO) has shown the neuroprotective effects, including anti-apoptotic and anti-inflammatory actions on central nervous system *in vivo* as well as *in vitro*.

Objectives: To evaluate the effects of EPO on the modification of disease progression and to ascertain most appropriate candidates for EPO therapy.

Methods: Fifty-seven patients with probable and definite ALS on the basis of El Escorial criteria were recruited for this study. After the lead-in period for 3 months to assess the progression of disease, recombinant human erythropoietin (rhEPO) was injected intravenously (35,000 IU/30min/50ml) four times at an interval of one month (0, +1, +2, +3 month) to rhEPO group. Patients were monitored by clinical evaluation including ALS Functional Rating Scale-Revised (ALSFRS-R), subjective symptoms and signs by 6 months follow up period. As a primary outcome measure, the change of ALSFRS-R score was compared between rhEPO (n = 21) and control (n = 20) groups. Additionally, the demographic and clinical characteristics in patients showing beneficial effects on EPO therapy were evaluated as a secondary outcome analysis. To assess the safety, patients were closely monitored for adverse events including thrombocytosis, malignant hypertension, and myocardial ischemia. Besides hemodynamic monitoring, each patient had complete blood cell count, blood urea nitrogen, creatinine, electrolytes, glucose and liver enzyme.

Results: When compared with the control group, the rhEPO treatment could reduce the change of ALSFRS-R during the first half follow up period. In addition, these disease modifying effects were enough to improve the functional deficits during 2 to 3 weeks after each rhEPO injection in 10 patients and more prominent in patients with short duration and rapid progression among the rhEPO group. The intravenous administration of rhEPO also had modest but significant beneficial effects including improvement of muscle power, decreased drooling, mood stabilization, and decreased spasticity. No significant adverse effects were noted. The hematocrit, hemoglobin, red blood cell counts, liver enzyme, and blood pressure remained stable.

Conclusions: Intravenous rhEPO in ALS is both safe and well tolerated. Moreover, this approach may be worthy of further investigation with various treatment interval and dosage.

P05 A PHASE II CLINICAL TRIAL USING THALIDOMIDE FOR THE TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS IS NOT EFFECTIVE

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Keywords: tumor necrosis factor-alpha, thalidomide, clinical trial

Background: Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease characterized by degeneration of upper and lower motor neurons. No treatment halts disease progression, which inevitably results in paralysis. Pathogenesis of motor neuron loss is likely initiated by genetic and/or environmental factors, but the mechanisms ultimately

remain unclear. Immune activation in the central nervous system (CNS) is postulated to play an important role. Information gathered from transgenic rodent models of ALS and human post-mortem studies implicates that the cytokine tumor necrosis factor – alpha (TNF-α) is involved and may be detrimental to motor neurons. In ALS patients and transgenic models serum levels and TNF-α soluble receptors are elevated compared to healthy controls. Increased TNF-α levels may induce apoptosis or disrupt axonal transport.

Thalidomide is a small molecular-weight pharmacological agent that penetrates the blood brain barrier. It has been shown to selectively reduce levels of TNF-α pre-transcriptionally and post-transcriptionally. It also inhibits angiogenesis by targeting basic fibroblast growth factor (bFGF). Thalidomide and other TNF-α antagonists have prolonged disease duration and extended the lifespan of transgenic animal models of ALS.

Objectives: An open-label, single-institution clinical phase II trial tested the efficacy of thalidomide in patients diagnosed with ALS. The primary endpoint in the trial was the ALS Functional Rating Scale. Secondary endpoints were, pulmonary function testing, survival stratified for newly diagnosed and progressive disease, toxicity, quality of life, and cytokine measurements. Controls from an existing ALS data base at another institution were used for statistical analysis.

Results: Twenty-three patients enrolled in the trial and were evaluated every 3 months for up to 9 months and in 2 cases longer. The major side effects of thalidomide included deep-vein thrombosis and peripheral neuropathy. Minor side effects included constipation, rash, sedation, bradycardia and elevated liver functions. Despite promising evidence from in vitro and in animal model studies, the trial was terminated early when we found that thalidomide had no statistically significant benefit on functional assessment scoring, survival, or pulmonary function. Serum cytokine analysis and post-mortem pathology are reported and discussed.

Discussion: The lack of efficacy may relate to any number of issues including the lack of appropriate receptor and intracellular targeting with thalidomide, a lack of efficacy related to an insufficient dosing, and the late initiation of therapy because of the inherent late diagnosis of the disease. Further clinical trials targeting TNF-α might be warranted, but this small trial does not provide support that reducing TNF-α levels improves ALS disease progression.

P06 CEFTRIAXONE TREATMENT IS ABLE TO REDUCE OXIDATIVE STRESS IN ALS PATIENTS

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Keywords: Oxidative stress, Glutamate, Ceftriaxone

Introduction: Glutamate-mediated neurotoxicity has been suggested as a mechanism of motor neuron death. Ceftriaxone has been considered putative therapeutic agent for ALS, by a modulation of astroglial GLT1 glutamate transporter expression.

Objectives: To report laboratory results of blood oxidative stress markers in ALS patients treated with a first course of ceftriaxone (Rocefin, 2g/day, for 14 days) within a planned one year study with bimonthly drug cycles.

Methods: The study has included 38 patients, 28 males and 10 females (mean age 62.2±SD 12.1-years). 14 patients presented with the bulbar form of disease, 10 the classic form and the remaining cases a predominant lower motorneuron onset form. Disease was evaluated by ALS-FRS-r, MRC scale, dynamometric test and respiratory functionally parameters as FVC, MIP and MEP. Blood levels of oxidative stress markers as glutathione (GSH), the advanced oxidation protein products (AOPP) and the ferric reducing ability of plasma (FRAP) were evaluated with colorimetric and spectrometric methods.

Results: Before therapy no correlation was found between oxidative stress marker blood levels, the duration and the severity of the disease. After ceftriaxone therapy AOPP levels decreased significantly ($p < 0.001$), while GSH and FRAP showed a slight not significant decrement. Respiratory functionally parameters, in particular FVC, were unchanged function.

Discussion: Based on the results, short term therapy with ceftriaxone is able to reduce circulating levels of some markers of oxidative stress in ALS. Whether or not this finding is antibacterial related to a direct effect of this drug on pathogenic mechanism of the disease or to an indirect effect mediated by its antibacteric action is still an open question, this is to be addressed with long term studies in conjunction with the assessment of the clinical effects.

P07 COMBINATION HIGH-THROUGHPUT SCREENING TO IDENTIFY EFFECTIVE THERAPEUTIC CHEMICAL COMBINATIONS TO UPREGULATE ASTROGLIAL PROTEIN EXPRESSION

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Keywords: astroglia, combination, EAAT2

Background: Glutamate is the predominant excitatory amino acid neurotransmitter in the mammalian CNS. Glutamate transporter, EAAT2 (or GLT1), is the physiological dominant astroglial protein to inactive synaptic glutamate. Animal studies show that EAAT2 dysfunction leads to excessive extracellular glutamate, and may contribute to various neurological disorders including amyotrophic lateral sclerosis (ALS), Huntington's disease, and growth of glioblastomas. Modulation of transporters may also be valuable in behavioral disorders such as depression. Through a recent screen of 1040 FDA approved drugs, we had revealed that beta lactam antibiotics protect against neural injury and delay disease in ALS mice through increasing GLT1 /EAAT2 expression. Thus, agents that upregulate the EAAT2 transporter might be beneficial in ALS and other neurological/psychiatric disorders by augmenting astrocytic uptake of glutamate. However, the beta lactam antibiotics had poor efficacy in increasing transporter protein.

Objectives: We proposed to employ a new drug discovery approach for identifying *effective and synergistic combinations* of compounds. Multi-component therapies through deliberate mixing of drugs have a successful history in a number of medical areas including HIV and oncology. Here we report our effort to develop a novel combinational high-throughput screening (cHTS) paradigm for identifying effective combinations of compounds to upregulate EAAT2 expression.

Methods: We generated fetal derived-human immortalized astroglial cells that are stably expressing a firefly luciferase reporter under the control of the human EAAT2 promoter (2.5 kb). Through cHTS of a library of up to 2000 FDA approved compounds and natural products, we discovered a serious of candidate drug-pairs to show synergistic effect to turn on the EAAT2 promoter. We have quantified synergy in

our screening experiments and identified combinations in which one drug enhances the potency or the intrinsic activity of the other drug. Follow-up analysis employed EAAT2 BAC luciferase reporter mice for whole animal validation of in vivo efficacy

Conclusions: One of our lead compounds, JR01, acts not only by increasing the intrinsic activity of its pair, but also mediating a shift in potency. We are further testing our leading candidates to see if they increase endogenous EAAT2/GLT1 expression in our *in vitro* and *in vivo* systems. Our studies provide potential neurotherapeutics by modulating the activity of astroglial proteins via gene activation.

P08 TREAT ALS PLATFORM: BRINGING STANDARDS AND EFFICIENCIES TO CLINICAL RESEARCH IN ALS

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Keywords: Clinical Trials Management System, TREAT ALS, Clinical Trial

Background: The Northeast ALS consortium (NEALS) was awarded a grant through The ALS Association's (ALSA) Translational Research Advancing Therapy for ALS (TREAT ALS) initiative to form the TREAT ALS/NEALS Clinical Trials Network with the goal to "translate research advances rapidly into clinical trials for patients with ALS".

Objective: To build a distributed Web-based clinical trial management platform to support the goals of the TREAT ALS initiative that will bring efficiency, scalability, regulatory compliance and standardization in conducting clinical trials and biomarker studies to ALS.

Methods: The TREAT ALS platform has been built on top of the existing PharmaContent system utilizing the "state machine" model for workflow configuration and customization; it is comprised of several modules that serve the following purposes: 1) To acquire and maintain the sites' information including contact data, sites' experiences with the TREAT ALS network, sites' IRB information and a list of equipment at each site. 2) To track the site and site members' regulatory documentation including professional licenses as well as compliance documents. 3) To study-specific workflow applications that help to expedite, regulate and standardize processes in clinical trials/studies management through using the following methods, i) Protocol module to track study protocols and protocol amendments at all participating institutions ii) Supply chain management module that allows sites and coordination centres to create orders of study supplies and track their status. iii) Financial module for automatic invoice generation for participating institutions based on either procedure- or visit-specific model. 4) Creation and maintenance of a bio-specimens inventory that includes samples from 15 clinical trials/studies. Samples may be searched upon by multiple criteria. 5) Standardization of data acquired from multiple NEALS studies and trials, which enables data analyses from multiple data sources.

Results: The TREAT ALS platform is currently deployed at the Neurology Clinical Trials Unit of MGH and is available to all member sites of the NEALS consortium. Four new investigators use this platform for their studies. Site and member information has been collected and is being maintained. The system issues regular notifications to the sites to update and upload expired and next-to-expiration regulatory documents. It became possible to track financial information for each study in real time knowing site payments and study supplies expenses.

Conclusion: Several new clinical research initiatives utilize the TREAT ALS platform. Developing a clinical trials management system for ALS studies adds efficiency and regulatory compliance to trials' conduct.

Acknowledgements: We thank The ALS Association for its continuing support.

P09 EXPLORING STOPPING RULES FOR ALS SCREENING TRIALS USING A BAYESIAN MODEL AND HISTORICAL PLACEBO DATABASE

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Keywords: futility, trial design, historical controls

Background: The vast majority of large clinical trials in ALS have failed, raising questions about how best to allocate resources for drug discovery. One option is to perform screening trials that avoid placing too many patients on an ineffective drug. Futility designs can play a role, but they allow only the most deleterious treatments to be stopped. Another way is to consider that most drug trials in ALS have failed and then to account for the likelihood that the chance that any given agent in the ALS pipeline will be effective is small. Under these constraints, even after a relatively small number of patients are tested, a response that looks similar to placebo would suggest a trial can be stopped.

Objective: To develop a Bayesian mathematical model, and use a database of historical placebo controls from three prior ALS trials, to explore the effects of different stopping rules for clinical trials. Understand how to predict likelihoods of drug efficacy after treating fixed numbers of patients in open label trials in ALS.

Methods: We created a Bayesian mathematical model that assumes a theoretical distribution of efficacies for potential agents in a pipeline of ALS drugs. Only a percentage of drugs will turn out to be effective and the prior probabilities of effect sizes of all drugs on the ALSFRS-R slope decline form a normal distribution with a mean of zero. We then calculated the posterior probability that a drug is effective, varying the number of patients treated (n, ranging from 30–100) and using means and standard deviations from a pooled distribution of 409 actual placebo patients from three previous phase III ALS trials, based on any observed outcome. We defined "effective" as a drug that reduces the slope by 20% or more. For the purpose of this study, we report results when the outcome is equivalent to historical placebo controls (decline in ALS-FRS of 0.83 units per month).

Results: We assumed a range of prior distributions where 5% of drugs tested would reduce ALSFRS-R slopes by 20% or greater; and a distribution where 20% of drugs reduce the slope by 20% or greater (the former probability roughly reflects outcomes of past ALS trials have been effective. The latter is a generous assumption that could reflect an upper limit for sensitivity analysis). Under the 5% assumption, an observed slope identical to historical placebo yielded a 2.5% probability that a drug will be effective with $n = 30$ patients. For $n = 60$ the probability was 1.3% and $n = 100$ was 0.5%. Using the 20% assumption, $n = (30, 60, 100)$, corresponding probabilities are 8.8%, 4.2% and 1.7%.

Conclusions: Considering the efficacy of drugs in the pipeline can help design stopping algorithms in ALS clinical trials. Bayesian modelling is a rational approach to trial design and

can show the small chance a drug will be effective after a small number of patients are treated. Decisions to stop trials are ultimately based on cost and understanding these probabilities. This can be contrasted with traditional statistics that avoid assumptions about the pipeline, and primarily focus on avoiding type II errors.

P10 PASSIVE IMMUNIZATION USING MONOCLONAL ANTIBODY SPECIFIC FOR MUTANT SOD1 DELAYED MORTALITY IN ALS G93A MUTANT SOD1 MICE

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Keywords: ALS, Passive immunization, monoclonal antibodies

Amyotrophic lateral sclerosis (ALS) is a fatal, yet untreatable, neurodegenerative disorder leading to motor neuron degenerartion, muscle atrophy, limb paralysis and death. There is emerging evidence for the existence of secretory pathways for superoxide dismutase (SOD1) mutants-linked ALS and for neurotoxicity of extracellular mutant SOD1. Active immunization paradigm has illustrated the potential to delay disease onset and mortality of ALS mice models. However, we believe that passive immunization approaches would be safer in future human ALS clinical tests. Thus, we tested a passive immunization approach through intraventricular infusion with miniosmotic pump, using conformation-specific antibodies against misfolded SOD1 mutant that we generated. The data show that it was also effective in alleviating disease symptoms and delaying mortality of ALS mice. From these results, we propose that immunization strategies, especially passive immunization, should be considered as potential avenues for treatment of familial ALS caused by SOD1 mutations.

P11 TARGETED LIPOSOMES FOR TREATMENT OF ALS IN MUTANT SOD1 MOUSE MODEL

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Keywords: liposome, targeted, minocycline

Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease hallmarked by the selective death of motor neurons accompanied by inflammation and microgliosis, the latter of which has been shown to have a neurotoxic role in the pathogenesis of the disease. To this end, several therapeutic agents have aimed to curb microglia activation, albeit with little success. To increase site-specific concentration and thus drug efficacy, we utilized a liposomal drug delivery system with targeting moieties specific to microglial cell surface markers to delivery the anti-inflammatory compound minocycline.

Objectives: The broad, long-term objective for our research is to develop a targeted nanotechnology platform that will improve treatment options for individuals suffering from ALS. Our central hypothesis is that minocycline contained in liposomes and delivered via intracerebroventricular (ICV) pump will delay disease onset and increase lifespan in mice carrying the Cu/Zn superoxide dismutase-1 (SOD1) mutation as compared to SOD1 mice receiving no treatment or sham pumps.

Methods: To test these hypotheses, we conjugated our liposomes with an LPS targeting moiety that is recognized by the Toll4 receptors on microglia. The drug is delivered continuously via an ICV osmotic pump that is implanted at 70 days of age. Efficacy is determined by the time to disease onset in treatment versus control groups, as well as the time to endpoint stages. Motor performance is measured with a rotarod apparatus with disease onset defined as the point in which a mouse can no longer stay on the rotating rod for >1 s.e.m. of the number of times it fell during the pre-symptomatic phase.

Results: Control mice ($n=7$) have an average disease onset at 107 days of age, whereas mice receiving targeted ($n=7$) and non-targeted ($n=6$) minocycline liposomes have an average disease onset at 113.4 ($p<0.05$) and 118.3 days ($p<0.001$), respectively. In addition, treatment groups experienced a similar increase in the time to endpoint stages (experiment ongoing).

Discussion and Conclusions: In the present study, we have shown that both disease onset and lifespan are significantly increased with our minocycline liposomal treatments. There appears to be little advantage to targeting the nanovesicles through an LPS peptide. Whether these data suggest minocycline is less effective if taken up by microglia will be pursued in future studies. Finally, this study serves as a proof of concept study for a drug delivery platform in which the "payload" of the nanovesicles can be easily modified to other compounds.

P12 EVALUATING THE EFFICACY OF SIMULTANEOUSLY DELIVERING IGF-1 AND VEGF EXPRESSING VIRAL VECTORS TO THE VENTRICULAR SYSTEM OF ALS MICE

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Keywords: neuroprotection, adeno-associated virus, trophic factors

Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that is characterized by a selective loss of motor neurones in the motor cortex, brain stem and spinal cord. Although a number of studies have demonstrated that trophic factors have potent effects on motor neuron survival their delivery to the CNS remains to be a challenge. Recently we showed that intraventricular delivery of trophic factor (i.e., IGF-1 and VEGF) expressing viral vectors resulted in trophic factor delivery to the CNS and improved survival in ALS mice.

Objective: In our current study we wished to determine if delivering IGF-1 and VEGF expressing viral vectors in combination to the ventricular system would lead to either an additive or synergistic improvement in efficacy.

Methods: Starting at 80 days of age (and every 10 days thereafter), $SOD1^{G93A}$ mice underwent behavioral testing (rotarod, hindlimb and forelimb grip strength) to assess motor function. At 90 days of age mice received either intraventricular injection of AAV4-IGF-I or AAV4-VEGF or a combination of both vectors. Control mice were injected with AAV4-GFP.

Results: We found that delivery of trophic factor (either IGF-1 or VEGF alone) expressing AAV4 vectors to the ventricular system significantly extended lifespan in ALS mice. Treatment with AAV4-GFP provided no survival benefit. Although a trend toward improved survival was observed in mice simultaneously treated with both IGF-1 and VEGF vectors, median survival between different trophic factor treatment groups was not significantly different.

Conclusion: Our results indicate that intraventricular injection of trophic factor expressing AAV4 vectors is an effective approach for slowing disease progression in a mouse model of ALS. Our findings also suggest IGF-1 and VEGF may be modifying disease progression in ALS mice by acting on similar biological pathways as co-administration of these trophic factors did not lead to a statistically significant enhancement in survival over treatment with either tropic factor alone.

P13 TREATMENT WITH A SOLUBLE ACTIVIN RECEPTOR TYPE LLB PROMOTES LEAN TISSUE GAIN AND IMPROVES GRIP STRENGTH IN SOD1G93A MICE

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Keywords: Myostatin, ActRllB, Muscle

Background: In amyotrophic lateral sclerosis (ALS), motor neurons responsible for innervating muscle progressively deteriorate. This muscle denervation results in muscle weakness and eventual atrophy. $SOD1^{G93A}$ mice express the mutant human Cu/Zn superoxide dismutase gene and therefore, serve as a rodent model of familial ALS. As in ALS patients, SOD1 mice display progressive muscle weakness and as the disease progresses, muscle atrophy and paralysis. Activin receptor type llB acts as the receptor for myostatin, a TGF beta family member which negatively regulates muscle mass, as well as other unidentified muscle mass inhibitors. RAP-031, a fusion protein comprised of the ActRllB extracellular region and an IgG domain, acts as a soluble receptor and inhibits endogenous ActRllB signalling, leading to significantly increased muscle mass. Previous work demonstrated that blocking myostatin lead to an improvement in the early stages of disease in $SOD1^{G93A}$ mice. This study evaluates the potential beneficial effects of RAP-031 treatment on muscle mass and strength in disease progressed $SOD1^{G93A}$ mice.

Objective: The aim of this study was to determine if treatment with a soluble activin receptor type llB can increase muscle mass and strength in $SOD1^{G93A}$ mice.

Methods: $SOD1^{G93A}$ mice already exhibiting signs of disease were divided by gender and further into treated vehicle or RAP-031 (once a week for 4 weeks) treated groups. Mice were assessed for grip strength and body composition at baseline and study end. At study completion, individual muscle groups were dissected and weighed.

Results: RAP-031 treatment increased body weight in both genders compared to vehicle treated groups. Both the male and female vehicle groups exhibited significantly decreased forelimb grip strength between study days 0 and 21 (male: −34.9%; female: −30.8%). At day 21, the male RAP-031 grip strength was also lower than its baseline values although to a significantly lesser extent (Male RAP-031: -12.9%). The RAP-031 treated female group maintained their baseline strength over the 21 days. The male vehicle group also exhibited a significant 6% lean tissue loss between the baseline and study day 28 measurements. In contrast, the RAP-031 cohort gained 9% of its baseline lean tissue mass. The female vehicle mice did not lose lean mass, but RAP-031 females increased total lean mass by 11%. At the end of the study, RAP-031 mice had significantly greater gastrocnemius, femoris and pectoralis weights compared to vehicle groups (male: 22.2%, 25% and 50%, respectively; female: 30.8%, 11.1% and 50%, respectively).

Discussion and Conclusions: These data demonstrate that RAP-031 increases body weight, lean tissue mass, individual muscle weights and improves grip strength in late-stage $SOD1^{G93A}$ mice and therefore could provide clinical benefit to ALS patients.

P14 METALLOTHIONEIN-IIA TREATMENT INCREASES SURVIVAL IN THE G93A SOD1 MOUSE

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Keywords: Metallothionein, G93A SOD1 mice, therapeutic

Background: Mutations to superoxide dismutase 1 (SOD1) enzyme have been linked to familial amyotrophic lateral sclerosis (ALS). These mutations typically cause a toxic gain-of-function resulting in tyrosine nitration and protein aggregation (1), mitochondrial dysfunction (2) and excitotoxicity (3). Mutant SOD1 also has a higher affinity to aggregate when zinc deficient (4). The neuroprotective protein metallothionein-IIA (MT-IIA) is involved in metal homeostasis (5), particularly that of zinc, and can act as an antioxidant (6).

Objectives: To test our predictions that MT-IIA may delay neurodegenerative decline and thus increase survival in the G93A SOD1 transgenic mouse.

Methods: At 10 weeks of age, litter pairs comprising a wild type and a mutant G93A SOD1 mouse, were injected intramuscularly into the left hindlimb with either MT-IIA or a saline control twice a week until the mice reached endstage (defined as a loss of 20% maximum body weight). At each injection time point, weight and disease symptoms (muscle wastage, hindlimb mobility and tremors) were assessed. Histopathological techniques are currently being used to assess the effect of MT-IIA treatment upon spinal motor neuron survival and integrity of the sciatic nerve.

Results: Symptom analysis showed a delay in disease development in the MT-IIA treatment group beginning at approximately 147 days of age. This finding can be attributed to the increase in survival seen in the MT-IIA treated group, which began at 145 days of age. Survival of the saline control group declined to 15% at 150 days of age while the MT-IIA treatment group still had 50% survival.

Conclusion: These results suggest that MT-IIA can prolong survival in the G93A SOD1 mouse. However, further research is required to determine the mechanism of neuroprotection by MT-IIA and its site of action.

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P15 AMMONIUM TETRATHIOMOLYBDATE, A COPPER-CHELATING DRUG, SUPPRESSES SOD1 AGGREGATION AND HAS THERAPEUTIC EFFECTS IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: SOD1 oligomer, Copper, Ammonium tetrathiomolybdate

Background: Amyotrophic lateral sclerosis (ALS) is an adult-onset fatal motor neuron disease. Toxic gain-of-function mutations in superoxide dismutase 1 (*SOD1*) are responsible for a subset of cases with familial ALS. Mutant SOD1 proteins form high-molecular-weight oligomers which tend to aggregate within the affected cells, such as the spinal motor neurons. A shift of the redox status of mutant SOD1 from the reduced form to the oxidized form is probably a key step in the aggregation process.

Objectives: To address the involvement of copper ions, which are known to possess redox properties, in the aggregation of mutant SOD1, we evaluated the effects of ammonium tetrathiomolybdate (TTM), a selective copper chelator, in a mouse model of ALS (G93A strain).

Methods: G93A mice were randomly assigned to receive a daily intraperitoneal administration with TTM (5 mg/kg) or phosphate buffered saline. Treatment was started at 4 weeks of age, before the G93A mice began to exhibit ALS-like symptoms. The clinical onset of the disease was evaluated by examining the mouse for shaking of its limbs when suspended in the air by its tails. The end-point was defined as the inability of the mouse to right itself within 30 seconds after being pushed onto it's side. The progression of the disease, duration of disease or survival after onset, was calculated as the period between the onset and the endpoint of the disease. For analysis of SOD1 oligomers, detergent insoluble fractions from spinal cords were suspended in Laemmli sample buffer without 2-mercaptoethanol. The fractions were electrophoresed, transferred onto a membrane, and immunoreacted with human SOD1 antibody.

Results: Immunoblotting showed that the removal of copper ions by TTM inhibited oligomer formation in the spinal cord. TTM also showed substantial therapeutic effects; it ameliorated ALS-like symptoms, including delaying of the symptom onset, slowing the progression and limiting the duration of the disease. Especially, survival was prolonged by approximately twofold as compared with that following treatment with riluzole, the sole approved drug for clinical use against ALS (11% for riluzole vs. 24% for TTM).

Conclusions: Copper ions are likely to contribute to oligomerization and aggregation of mutant SOD1. TTM may be a promising drug for familial ALS.

P16 SIGNALS IN PATHOLOGICAL MICROENVIRONMENT OF ALS MICE PROMOTE hMSCs NEUROGENIC DIFFERENTIATION IN VITRO

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Keywords: Differentiation; mesenchymal stem cells; amyotrophic lateral sclerosis

Background: Amyotrophic lateral sclerosis (ALS) is a progressive, lethal, neurodegenerative disease, currently without any effective therapy. Multiple advantages make mesenchymal stem cells (MSCs) a good candidate for cellular therapy in many intractable CNS diseases. But some studies have cast doubt on neuronal differentiation of the MSCs using non-physiological chemical inducing agents *in vitro* which are apparently different from physiological or pathological state *in vivo*.

Objectives: This study was designed to use conditioned medium to investigate whether signals from pathological state of ALS were competent to induce a program of neurogenic differentiation in expanded cultures of hMSCs and to assess their therapeutic potency in ALS.

Methods: hMSCs were isolated from iliac crest aspirates from healthy donors and kept in cell cultures. Conditioned medium of SOD1-G93A mice was made. The morphological change of hMSCs and the expression of neuronal markers were analyzed.

Results: We observed that incubation of hMSC with conditioned medium prepared from CNS of SOD1-G93A mice resulted in a time-dependent morphological change from fibroblast-like into neuron-like cells, concomitant with increase in the expression of nestin and subsequent β-TubIII, NSE and Gap43. Moreover, signals in pathological environment of ALS mice were more effective in promoting the neurogenic differentiation of hMSCs than in the physiological environment of CNS in adult mice.

Conclusion: These results show that pathological microenvironment of ALS is endowed with capacity to induce neurogenic differentiation of hMSCs and hMSCs have shown a potential candidate in cellular therapy for ALS.

P17 OPTIMAL MESENCHYMAL STEM CELLS FOR CLINICAL USE IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Keywords: Mesenchymal stem cell, cytokine, growth factor

Background: Amyotrophic lateral sclerosis (ALS) is a rapidly progressive, fatal, neurodegenerative condition characterized by loss of upper and lower motor neurons in brain and spinal cord. At present, riluzole, a glutamate antagonist, remains the only effective therapy, but with only minimal effects on survival. Therefore, stem cell research has highlighted ALS as a target disease for stem cell treatment. As a source of stem

cell therapy, it has been reported that Mesenchymal Stem cells (MSCs) display neuroprotective effects and could influence on disease progression in ALS. Considering the low density (0.01% to 0.001%) in bone marrow, *ex vivo* expansion of MSCs is necessary prior to clinical use. However, the stability and characterization of MSCs during long-term *in vitro* expansion have not been evaluated yet.

Objectives: To evaluate the optimal passage for autologous MSCs therapy in ALS patients.

Methods: MSCs from bone marrow of ALS patients were isolated and expanded. The cumulative population doublings, specific antigen expressions, karyotype, differentiation potential, and the expression of various cytokines and growth factors in successive passages were evaluated.

Results: During long-term culture, the size and the morphology of the MSCs transferred from small and spindle-like cells to large and polygonal types although the specific antigenic expressions for MSCs were shown in late passages. Population doublings were gradually decreased when passages went on. MSCs at fourth passage were induced to mesenchymal lineage cells, including adipocytes, osteocytes, and chondrocytes. When we analyzed the cultured media of MSCs at third, fifth, seventh, and ninth passages, IL-6, VEGF, and IL-8 showed high expression of more than 50 pg/10,000 cells in all these passages but their expression rate was continuously decreased. Additionally, secretion of IL-15, GM-CSF, IL-10, PDGF bb, G-CSF, IL-1β, FGF basic, and IFN-gamma was gradually decreased as culture time increased.

Conclusions: It is clear from our studies that MSCs gradually become large and shows the decrease of growth rate, differentiation potential, and cytokine release during long-term culture. Therefore, we might suggest that MSCs at earlier passage would be suitable for stem cell therapy considering its stability and neuroprotective roles associated with various cytokines and growth factors.

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THEME 2 IN VIVO EXPERIMENTAL MODELS

P18 MESENCHYMAL STEM CELL TRANSPLANTATION IN AN ALS MOUSE MODEL

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Keywords: transplantation, mesenchymal stem cell, transgenic mice

Background: ALS selectively affects motor neurons throughout the central nervous system (CNS) from the cerebral cortex to the lumbar spinal cord. This wide distribution of motor neurons is an obstacle in applying cell transplantation therapy for the treatment of ALS. We developed a transplantation protocol that enables transplanted cells to broadly diffuse throughout the CNS. We applied this method to the treatment of an ALS mouse model.

Objectives: To examine whether the transplantation of mesenchymal stem cells (MSCs) into the CNS shows any beneficial effect on clinical or pathological aspects of disease. The use of MSCs is advantageous since they have the capability to differentiate into several specific cell types including neural cells; furthermore, they can be relatively easily obtained by biopsy in humans, thus making autologous transplantation possible.

Methods: MSCs were derived from 5-week-old rats that ubiquitously express green fluorescent protein (GFP). As the host we used $SOD1^{Leu126delTT}$ transgenic mice which express a mutated form of SOD1 with a 2-bp deletion at codon 126. The MSCs were transplanted into 100-day old $SOD1^{Leu126delTT}$ transgenic mice via the fourth ventricle and a control group of mice received a sham operation. From one week prior to the transplantation until death occurred, clinical evaluations of body weight, hind limb extension reflex score, and footprint analysis were performed once a week. The MSCs in the host spinal cord were pathologically observed by immunofluorescent staining of frozen sections.

Results: There were beneficial tendencies in age of onset, death, and disease duration in MSC-transplanted mice compared to control mice, but these did not reach statistical significance. However, when we compared only MSC-treated female mice with sham-operated female mice, we could find a statistically significant difference for disease duration between the two groups (MSC; 21.6 ± 5.4 days vs. control; 10.0 ± 2.2 days). On pathological examination, we confirmed the existence of transplanted MSCs in the anterior horns of transgenic $SOD1^{Leu126delTT}$ animals. Although there was a trend that the MSC group's motor neurons out numbered those of the sham group, the remaining motor neuron count immediately before death was similar between MSC transplanted animals and sham operated animals.

Conclusions: MSCs modestly prolong disease duration in female transgenic $SOD1^{Leu126delTT}$ animals. Therefore, our transplantation method could be a promising way to deliver donor cells to the CNS. Further experiments to optimize timing and frequency of the cell transplantation are necessary. Additionally, it might prove effective to use genetically modified cells expressing a growth factor, such as the glial cell line-derived neurotrophic factor or insulin-like growth factor.

P19 DIGESTION OF THE EXTRACELLULAR CHONDROITIN SULFATE PROMOTES AN INTRINSIC REGENERATIVE PROCESS IN THE SPINAL CORD OF ALS TRANSGENIC RATS

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Keywords: SOD transgenic microenvironment

Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by adult-onset selective motor neuronal loss. Approximately 2% of all ALS cases are linked to mutations in the Cu/Zn superoxide dismutase (SOD1) gene. Upregulation of extracellular chondroitin sulfate proteoglycans (CSPGs) under various types of CNS insults is known to restrict intrinsic regenerative processes such as neurite outgrowth, synaptogenesis and cell migration.

Objectives: In order to clarify the role of extracellular CSPGs under neurodegenerative condition such as ALS, we tested the effect of enzymatic degradation of CSPGs in the spinal cord of a transgenic rat model of ALS.

Methods: We examined the expression of CSPGs in the lumbar spinal cord of His46Arg mutant *SOD1* transgenic ($SOD1^{H46R}$ Tg) rats at presymptomatic, early symptomatic, and late symptomatic stages with their age-matched non-transgenic (non-Tg) littermates. Based on the results, a bacterial enzyme chondroitinase ABC (ChABC) was continuously infused into the subarachnoid space of mid-symptomatic $SOD1^{H46R}$ Tg rats for 7 days. Over the same period, we also infused a thymidine analogue bromodeoxyuridine subcutaneously to label the newborn cells *in vivo*. After the infusion, we performed immunohistochemical analysis in the lumbar spinal cord to confirm the effect of ChABC. In addition, we compared the neuropathology, proliferative neural progenitors, and synaptogenesis in the spinal ventral horns between ChABC-treated and vehicle-treated $SOD1^{H46R}$ Tg rats.

Results: In contrast to non-Tg rats, the $SOD1^{H46R}$ Tg rats showed significant and progressive upregulation of CSPGs in the ventral spinal cord even from presymptomatic stage. In the spinal cord parenchyma of ChABC-treated $SOD1^{H46R}$ Tg rats, immunohistochemistry revealed significant reduction of the chondroitin sulfate deposition as compared with vehicle-treated rats. In addition, bromodeoxyuridine-incorporated newborn cells including immature phenotype, synaptophysin-positive structures, and neurons with immature phenotype were significantly increased in the ventral horns of ChABC-treated rats. On the other hand, neuropathology such as loss

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of ventral horn neurons, ubiquitinated protein aggregates, and phosphorylated neurofilament accumulation was not exacerbated in the ChABC-treated rats.

Discussion and Conclusions: In the present study, we found significant and progressive deposition of CSPGs in the spinal cord of transgenic rat model of ALS. Although a possible neuroprotective role of CSPGs remains to be argued, the present results suggest that the excessive deposition of CSPGs may play an inhibitory role against intrinsic regenerative processes under the ALS-like disease in the present model. Therefore, regulation of extracellular molecules in the microenvironment surrounding motor neurons could be an important strategy to develop cell-restorative therapy in ALS.

P20 EFFECTS OF EDARAVONE, A FREE RADICAL SCAVENGER APPROVED IN JAPAN FOR INDICATIONS OF ACUTE ISCHEMIC STROKE, IN A TRANSGENIC RAT MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: H46R SOD1 transgenic rat, free radical, Edaravone

Background: The toxic gain-of-function of mutant SOD1 enhances oxidative activity, produces hydroxyl radical or peroxynitrite has previously been hypothesised. Therefore free radicals are presumed to contribute to the pathogenesis of ALS.

Objectives: We investigated the efficacy of edaravone, a free radical scavenger previously approved for acute ischemic stroke in Japan, using a rat model of ALS expressing a human SOD1 transgene with ALS associated mutation: histidine to arginine at position 46 (H46R).

Methods: H46R SOD1 transgenic rats of both sexes were used in this experiment.

Edaravone (1.5 and 3.0 mg/kg/h, male: n = 8, female: n = 8, in each group) and saline (male: n = 8, female: n = 8) were administered intravenously to rats by continuous infusion (1 hour infusion per day). Two days of administration was followed by 2 days of drug holiday. This series commenced from when the rats were 18 weeks old and was repeated until the day of loss of righting reflex.

The lifetime and duration of illness were evaluated, and besides these, motor function was assessed using expansion of limbs by tail suspension (suspension test), width between hind limb when landing from 30 cm height (landing test), rota rod test and inclined plate test.

The number of motor neurons of the third lumber spinal cord in the vehicle group and edaravone 3.0 mg/kg/h group were also examined at the time point of 24-weeks old.

Results: We defined the onset of disease as the day of the observation of postural change in one limb when the rat was lifted by the tail (suspension test). The death of animal was defined as the day when the righting reflex disappeared.

Lifetime of saline treated, edaravone low dose and edaravone high dose group was 196 ± 3, 200 ± 1 and 202 ± 4 days respectively, in males and 212 ± 4, 214 ± 5 and 224 ± 10 days, respectively in females. Duration of illness of saline treated, edaravone low dose and edaravone high dose group was 52.0 ± 3.2, 54.3 ± 2.1 and 57.6 ± 5.2, respectively, in males and 67.6 ± 4.6, 72.1 ± 4.6 and 78.7 ± 10.5 respectively, in females. Edaravone elongated both lifetime and duration of illness according to the dose escalation, however, did not reach significance.

Evaluation point of motor function was set up on the day that half of the saline-treated animals died. In suspension test, rota rod and inclined plate test, high dose of edaravone tended to improve both sexes, especially in landing test where a high dose of edaravone treated male group showed a significant improvement.

No difference was observed in number of motor neurons between the vehicle group and the edaravone treated group at the time point of 24 weeks old.

Discussion and Conclusions: In this study, we demonstrated the possibility of edaravone as a therapeutic agent for ALS using H46R SOD1 transgenic rat. Further studies are needed to confirm the efficacy of edaravone in the near future.

P21 MELATONIN IS DETRIMENTAL TO SURVIVAL IN A TRANSGENIC MOUSE MODEL OF FAMILIAL ALS

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Keywords: Melatonin,G93A, mice

Background: There is experimental and human data that reactive oxygen species (ROS) and apoptosis are involved in the pathogenesis of amyotrophic lateral sclerosis (ALS). In familial ALS due to mutations of the Cu/Zn superoxide dismutase (SOD1) it is postulated that the mutated SOD1 exhibits a novel gain of function and acts as a supplier rather than a scavenger of ROS. In G93A SOD1 transgenic mice there is an enhanced production of ROS in the spinal cord, which precedes motor neuron degeneration. Melatonin is an endogenous neurohormone with well recognized anti-oxidant and anti apoptotic properties, both as a direct ROS scavenger as well as by up regulating anti-oxidant enzymes. Melatonin has also been shown to protect neurons from kainate induced glutamate-receptor excitotoxicity and to attenuate lipid peroxidation and microglial activation.

Objective: To assess the effect of melatonin on disease phenotype in the G93A-SOD1 transgenic mouse model of ALS.

Methods: Four groups of 10 mice each, carrying the human G93A-SOD1 mutation (BS6JL-TgN SOD1-G93A), were injected with 0 mg/kg, 0.5 mg/kg, 2.5 mg/kg and 50 mg/kg of melatonin intraperitoneally from the age of 40 days. The end points were disease onset, survival, rotarod performance and morphometry of lumbar cord motor neurons. SOD1 expression was studied with histochemistry, Western blot and Real Time PCR.

Results: There was no statistically significant difference in disease onset between the four groups although there was a

tendency for the 2.5mg/kg dose to delay onset. Survival was significantly reduced ($P < 0.05$, two tailed t-test) with the 0.5mg/kg and 50mg/kg doses and tended to be reduced with the 2.5mg/kg dose. Histochemistry against SOD 1 revealed higher expression of the protein in motor neurons of mice treated with melatonin. Western blot and Real Time PCR revealed a dose dependent up regulation of SOD1 in melatonin treated animals.

Conclusion and Discussion: Melatonin, contrary to expectations, reduces survival in the G93A mouse model of ALS despite its known anti oxidant and anti-apoptotic properties. This phenomenon is probably accounted for by the up regulation of anti-oxidative enzyme gene expression including, in the G93A transgenic mice, the mutated and toxic human SOD1. This action of melatonin probably overrides any of its beneficial effects. This study reinforces the notion that, at least in SOD1 associated ALS, ROS play a pathogenic role.

P22 THE CHICK EMBRYO AS A TOXICITY SCREEN FOR GENES IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: Chick, SOD1, TDP43

Background: Genetic technology has advanced to the point that large numbers of people are being tested for gene variants contributing to ALS. The chick embryo provides a model system in which effects on intact tissues can be examined quickly and simply in a cell and stage-specific way. We have used the chick embryo model in order to study the effects of gene mutations found by linkage studies.

Methods: We electroporated $SOD1^{G93A}$-dsRed, $SOD1^{WT}$-dsRed and HA and Myc tagged TDP-43^{WT}, TDP-43^{Q331K} and TDP-43^{M337V} DNA into the spinal cords of HH stage 14 chick embryos using *in ovo* electroporation. After 24 hours the embryos were processed for frozen sectioning and then apoptotic cell death was observed using TUNEL assay. Immunohistochemistry of neuronal markers was carried out in order to investigate the types of neurons affected.

Results: $SOD1^{G93A}$-dsRed transfected neurons showed cell death as demonstrated by TUNEL staining (no. of embryos analysed = 36/36). We also observed a decrease in the population of motor neurons (stained for Islet1/2) on the transfected side of the embryo as compared to the non-transfected control side.

Embryos expressing mutant TDP43 (n = 49) showed a dramatic reduction in maturation as observed by a failure to develop normal limb and tail buds. The percentage of mature embryos electroporated with TDP-43^{Q331K} was 11.66% and for TDP-43^{M337V} was 15% as compared to 97.66% for embryos electroporated with TDP-43^{WT}. TUNEL staining demonstrated a significant increase in the number of apoptotic nuclei in embryos expressing either mutant TDP-43 when compared to TDP-43^{WT} (TDP-43^{Q331K} n = 29.5; TDP-43^{M337V} n = 21.6, TDP-43^{WT} n = 5.6, where n is mean of TUNEL positive cells/section for 5 sections analysed from 3 embryos).

Conclusions: The chick model offers a relatively quick and cost-effective means of validating genetic discoveries and will provide valuable insights into disease mechanisms.

P23 NEUROTOXIC SPECIES OF MISFOLDED MUTANT SOD1 DISPLAYING CROSS-REACTIVITY WITH ANTIBODIES AGAINST P2X4 SUBUNIT OF THE ATP RECEPTOR ACCUMULATE IN DEGENERATING NEURONS BUT NOT IN GLIAL CELLS OF TRANSGENIC $SOD1^{G93A}$ RODENTS

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Keywords: SOD1, P2X4, neurodegeneration, microglia

Background: We have recently described that degenerating motor neurons (MNs) in spinal cord, brainstem and cerebral cortex of $SOD1^{G93A}$ rats or mice, exhibit an intense immunoreactivity to P2X4 antibodies (1). Neurons with strong P2X4-like immunoreactivity (P2X4-LI) are often associated with microglial cells displaying neuronophagic activity and do not show apoptotic phenotype when dying. Degenerating neurons with strong P2X4-LI and microglial cell recruitment were also observed in the cerebellar cortex and in noradrenergic or serotoninergic systems of the brainstem. After Western blot analysis it was found that P2X4 antibodies recognize, in addition to P2X4-membrane associated protein, an unidentified low MW band seen in cytosolic extracts from $SOD1^{G93A}$ but not in wild type (WT) samples.

Objectives: To identify of the molecular counterpart of the strong P2X4-LI observed in association of neuronal degeneration and death in $SOD1^{G93A}$ animals.

Methods: Soluble spinal cord extracts were subjected to 2D electrophoresis and P2X4 immunoreactive spots were analyzed by proteomics. Further studies include: immunoprecipitation, multiple fluorescent labelling of tissue sections and confocal microscopy.

Results: On Western blots of spinal cord extracts, we have found that the antibody recognizes a protein band in membrane fraction with the expected size of P2X4 receptor protein (40–60 kDa) that is upregulated in end-stages of SOD1 G93A rats. After MALDI-TOF it was found that the low MW, P2X4-immunoreactive (P2X4-IR) protein band seen in the cytosolic fraction of transgenic animals was SOD1. This result was further confirmed by immunoprecipitation experiments showing that anti-P2X4 antibody is able to immunoprecipitate SOD1 and that anti SOD1 antibody immunoprecipitates a P2X4-IR band migrating at the level of SOD1. By double labelling immunohistochemistry it was found that P2X4-LI in spinal cord tissue sections partially overlaps with SOD1 in spinal cord from $mSOD1^{G93A}$ mice. Whereas in $mSOD1^{G93A}$ samples SOD1 immunostaining was present in both neurons and glia, P2X4-LI was only detected in degenerating neurons, being absent in spinal cord tissue sections from mice overexpressing human WT SOD1. P2X4 antibodies were able to immunoprecipitate erythrocyte-derived unfolded SOD1 more efficiently than the folded one. Affinity chromatography-isolated P2X4-LI SOD1 species were injected intracerebrally in mice in order to examine its pro-inflammatory properties; preliminary results indicate that microglial activation by P2X4-LIR-$SOD1^{G93A}$ was higher than that by human WT SOD1, used as a control. Studies on P2X4-LI in NSC34 motor neuron cell line expressing mutant SOD1 are in progress.

Discussion and Conclusions: From these results we deduce that P2X4 antibody recognizes a form of misfolded mutant SOD1 which is expressed only in neuronal cells undergoing degeneration. It seems that the cross-reactivity could be due to the abnormal exposure of hydrophobic regions of SOD1

sharing structural homology with the P2X4 immunizing peptide that was used for raising the antibody.

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P24 SOLUBLE MISFOLDED SOD1 IN TRANSGENIC MICE IS MOSTLY OLIGOMERIC

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Keywords: Misfolded SOD1, Oligomers, Disulphide reduction

Background: 140 different mutations in SOD1 have been found in ALS cases and all should share a common neurotoxic mechanism. A common denominator of transgenic mice expressing various mutant SOD1s, is enrichment of soluble misfolded forms of the protein in the vulnerable spinal cord (1).

Aims: To determine the molecular structure of misfolded SOD1 in murine spinal cords. Antibodies specific for misfolded forms of SOD1 were used for the purpose.

Methods: Sandwich ELISAs (misELISAs) were developed using antibodies specific for misfolded SOD1 and directed at different parts of the molecule. Tissue extracts from transgenic mice were subjected to gel chromatography. Eluting fractions were analyzed for total SOD1 with Western immunoblots. Misfolded SOD1 in the fractions was analyzed with the misELISAs. The disulphide status of SOD1 was determined with non-reducing Western immunoblots (2).

Results: In G93A transgenic mice two peaks were seen in the chromatography when analysed for total SOD1. One large peak of 32 kDa composed of dimeric SOD1 and a smaller of 16 kDa with monomeric SOD1. The misELISAs showed two peaks, one at 140 kDa and one at 45 kDa. This would correspond to SOD1 nonamers and trimers. Less than 2% of the total SOD1 was found to react in the misELISAs. Analysis of D90A and G85R mice with the misELISAs show similar nonamer-trimer patterns with an additional small monomeric peak. The oligomeric SOD1 was not disulphide-coupled and lacked the disulphide bond.

Discussion: In many neurological diseases large protein aggregates are found late in the disease process. If the aggregates are toxic or if precursor molecular forms exert the toxicity is not known. We show here that misfolded SOD1 in tissues adopts several conformations including monomeric, tri and nonameric forms. Whether these are toxic remains to be shown.

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P25 BIOCHEMICAL CHARACTERIZATION OF HUMAN G93A SOD1 MUTANT PURIFIED FROM TRANSGENIC RAT SPINAL CORD

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Background: Mutations to SOD1 remain the only proven cause of ALS, but the mechanism whereby mutants cause neurotoxicity remains unknown. *In vitro* studies with recombinant SOD1 mutants have been shrouded in controversy as to which properties are "real" and which are related to the necessarily artificial conditions used to express and purify them. To address this question, we sought a way to quickly and effectively purify SOD1 from transgenic rat spinal cord using very mild conditions, so that the true "as isolated" properties of a disease-causing mutant could be unequivocally established.

Objective: To compare properties of the human G93A mutant enzyme purified from spinal cord of both presymptomatic and endstage transgenic rats with those of recombinant G93A obtained from *E. coli*. Determination of the properties of G93A "as isolated" from spinal cord may provide clues as the toxic mechanism of SOD1 mutants, and allow us to better assess the suitability of recombinant enzymes to test various toxic hypotheses.

Methods: Spinal cords were harvested from 65 day old and endstage transgenic rats and homogenized, clarified, and the supernatants injected into a polymeric, reversed-phase preparative HPLC column maintained and eluted at pH 7.4 (Tris with linear acetonitrile gradient). The fraction corresponding to native homodimer was collected and overloaded onto an SDS-PAGE gel to assess purity. For each sample ($n = 3$ for each condition), Zn, Cu, and reduced thiol content were determined, and normalized to enzyme subunit based on rigorous determination of protein concentration. Specific dismutase activity was also determined.

Results: SDS-PAGE gels revealed that the one-step HPLC purification procedure yielded $>99\%$ pure G93A from rat cord within 20 minutes of tissue homogenization. Analysis of pure enzyme revealed $\sim 50\%$ of the maximal content of Zn and Cu. Reduced thiol (-SH) content of native enzyme was ~ 0.2/subunit, and increased to ~ 1.2 upon denaturation (the same was seen with recombinant G93A). Specific activity of rat cord G93A was $\sim 4{,}000$ units/mg, however, because the Cu content was only 50% of maximal, the true specific activity is arguably $\sim 8{,}000$ units/mg of copper-saturated enzyme.

Discussion: The results are consistent with the surface accessible cysteine (Cys111) existing in the oxidized state, whereas Cys6 is reduced. The ratios of Zn and Cu per subunit are consistent with one metal ion per dimer. Limiting amounts of CCS and Cu could explain the low Cu levels (when SOD1 is grossly overexpressed), but this would not explain the low levels of Zn. With the exception of somewhat lower Zn and Cu content, G93A from rat cord was essentially identical to enzyme obtained from *E. coli*, suggesting that recombinant enzymes are, indeed, suitable for examining toxic properties, particularly when wild-type enzyme is prepared using identical conditions.

P26 TRANSCRIPTIONAL ANALYSIS BY SUPPRESSION SUBTRACTIVE HYBRIDIZATION OF TEMPORAL AND CONDITIONAL CHANGES IN SPINAL CORD OF THE SOD1 G93A MOUSE MODEL OF FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: subtractive hybridization, SOD1 mutation, differential gene expression

Background: Mice transfected with the human SOD1 mutation G93A develop amyotrophic lateral sclerosis (ALS) like symptoms at approximately 100 days of age. Biochemical changes in motor areas in these mice will provide clues as to the cause and possible treatment of this condition in human patients. Using the procedure of suppression subtractive hybridization (SSH) the transcription events before and after the onset of symptoms (temporal comparison) and between normal and SOD1 mice at the two ages tested (conditional comparison) provide valuable information as to the general metabolic events taking place in the spinal cord during this process.

Objectives: To compare normal (C57Bl6J) and hemizygous (SOD1-G93A) mouse spinal cords at 60 (pre-symptomatic) and 120 (post-symptomatic) days of development for changes in transcribed expressed sequence tags (ESTs). Direct comparisons are between SOD1 and normal mice at 60 and 120 days (conditional) and between normal mice at 60 and 120 days and SOD1 mice at 60 and 120 days (temporal) resulting in eight reciprocal comparisons.

Methods: Total and messenger RNA was isolated from spinal cords dissected from mice and immediately frozen on dry ice. Direct comparisons of cDNA derived from these preparations were compared by SSH in reciprocal subtractions. Subtraction efficiencies between 5 and 10 cycles only were used for comparisons and clones derived from these sequences were further selected by dot blot assay. Representative differentially expressed genes were ultimately verified by northern analysis.

Results: Over 600 sequences were compared between these eight conditions. No differentially expressed sequences were observed in any reciprocal subtractions indicating complete separation during these subtractions. A control subtraction of SOD1 60 day animals against normal 60 day animals without spiking out human SOD1 sequences, showed in excess of 75 percent of these sequences, indicating the power of this procedure. A large percentage of the sequences were either ESTs of unknown function or represented by to-date unrecorded transcripts making analysis of any influence on metabolic pathways difficult (the majority of unknown sequences were in the temporal comparisons rather than in the conditional comparisons). However, there is an obvious predominance of myelin sequences in younger SOD1 mice when subtracted against younger normal mice and a similar preponderance of these sequences in younger normal mice. Animals carrying the G93A SOD1 gene also showed a preponderance of structural and respiratory associated sequences when compared to normal controls.

Discussion and Conclusions: Overall patterns of expression indicate that SOD1 mice compensate for disturbances in oxidative processes in the pre- symptomatic animals. Normal and SOD1 animals both show a preponderance of structural and myelin sequences at earlier ages and a dominance of unknown and unrecorded ESTs at later ages. Results of this study are compared and contrasted to other studies using similar specimens and comparative transcription procedures.

P27 GENETIC MODIFIER AND PHENOTYPIC ANALYSIS OF Tg hSOD1 G93A CONGENIC MOUSE STRAINS

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Keywords: hSOD1 G93A, modifier genes, QTL

Background: Mutations in the gene encoding superoxide dismutase 1 (SOD1) cause amyotrophic lateral sclerosis (ALS) in humans. Studies of SOD1-induced ALS frequently employ a transgenic mouse model that over-expresses the human mutant form of SOD1 that has a glycine replaced by an alanine at position 93 (hSOD1-G93A). We investigated the influence of genetic background on hSOD1-G93A-induced ALS by breeding the transgene onto several inbred mouse strains to create fully congenic lines. Our results show that genetic background can significantly modify survival time. We selected two congenic SOD1 strains, C57BL/6J and ALR/LtJ for further investigation. B6.SOD1 mice live nearly two months longer than ALR.SOD1 mice. A mapping cross between these two strains identified a major B6-dominant modifier located on Chr. 17 and a slightly less significant B6-dominant modifier located on Chr. 4.

Objective: 1) To compare the phenotypic expression of the ALS-induced disease in the two congenic strains to identify differences that might contribute to the earlier disease onset. 2) To analyze candidate genes in the QTL regions that have previously been associated with ALS in humans.

Methods: Mice from each strain were age-matched relative to their 50% strain survival time and characterized at 3 time points. Phenotypic measures included innervation status, peripheral axon loss, gait analysis, muscle contractile force, and immunoblot analysis of SOD1 positive aggregates in the spinal cord. Initial examination of candidate genes was performed using RNA and cDNA from spinal cord tissue. Nucleotide sequence of candidate genes was examined for polymorphisms between B6.SOD1 and ALR.SOD1 and quantitative PCR was used to look for expression differences between candidate genes.

Results: To date phenotypic characterization has revealed no significant differences between B6.SOD1 and ALR.SOD1 mice age-matched relative to their 50% survival. The two strains show similar: 1) presymptomatic modification of gait, 2) loss of innervation at the neuromuscular junction, 3) decreases in axon counts of muscle nerves and 4) loss of contractile force. Data collection for immunoblot analysis and data from additional time points for other measures remain to be completed.

There are no major alterations in splice forms, sequence polymorphisms, or expression levels for Tardbp between B6 and ALR. Preliminary results indicate that there are several sequence polymorphisms in DAXX between the strains, and these are being further characterized. A further candidate gene, Mapk14, is also being analyzed.

Conclusions: Phenotypic characterization completed to date has not revealed any differences that predict the accelerated

onset of the hSODG93A induced ALS between the strains. Candidate genes in the intervals are presently being further tested for sequence differences. Gene chip expression analysis is being initiated to discover other potential candidate genes.

P28 TIME-COURSE OF BULBAR V. SPINAL MOTOR DEFICITS IN MALE AND FEMALE SOD1-G93A MUTANT RATS

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Background: Amyotrophic lateral sclerosis (ALS) is a progressive degenerative disease of upper and lower motor neurons with an invariably fatal outcome. Symptom onset may occur in the muscles of the limbs (spinal onset) or those of the head and neck (bulbar onset). Although the pathogenesis of sporadic ALS is unknown, mutations in the Cu/Zn superoxide dismutase-1 (SOD1) gene are associated with the familial form of ALS (fALS). Several transgenic rodent models of fALS have been developed, with the SOD1-G93A being the most widely studied. These animals develop an ALS-like phenotype that includes muscle weakness and atrophy, and degeneration of motor neurons in the cortex, brainstem and spinal cord. Spinal involvement has been the focus of most preclinical studies to date. Recently, we documented behavioural bulbar involvement in SOD1-G93A mice (1) and wished to extend this characterization to SOD1-G93A rats.

Objectives: The purpose of this study was to determine the relative onset of bulbar v. spinal motor signs in male v. female SOD1-G93A rats.

Methods: In the clinic, tests of bulbar function include evaluation of tongue strength and motility. Here, we measured orolingual motor function by placing water-restricted rats in a customized operant chamber that allows for the measurement of tongue force (g) and licking rhythm (licks/s) as animals lick water from an isometric disc. Rats were tested 3 times/week for over 200 days, spanning the pre-symptomatic, symptomatic, and end stage segments of the disease. Peak tongue force and licking rhythm were recorded during the 6-minute test sessions. Body weight and fore- and hindlimb grip strength were also monitored concomitantly. Rats were killed within 7 days of onset of overt paralysis in at least 1 limb.

Results: Training-related increases in tongue motility were attenuated in affected rats from ~40–90 days of age. Licking rhythm continued to be attenuated in the SOD1-G93A group and primarily in females throughout the experiment. Tongue force was not affected. In female SOD1-G93A rats, body weight gain diminished compared to wildtypes beginning ~ 140 days of age, while weight gain in affected males diverged from healthy males at 180 days. Hindlimb grip strength deficits emerged at 6 months of age in both males and females.

Conclusions: To our knowledge, these findings are the first to detail the onset of bulbar v. spinal motor signs in this preclinical model of ALS. It is apparent that our rats were low-expressing SOD1-G93A transgenics. Our results suggest that bulbar symptoms emerge primarily in female SOD1-G93A rats and prior to spinal symptoms. Our results also suggest that water restriction may reveal sexually dimorphic effects in the rat SOD1-G93A model of ALS. A detailed characterization of bulbar involvement will be important for future evaluation of therapeutic agents.

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P29 GENDER DIFFERENCES IN SURVIVAL OF G93A SOD1 MICE IS NOT DUE TO DIFFERENCES IN MUTANT SOD1 EXPRESSION

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Keywords: SOD1,G93A SOD1 Transgenic mouse,phenotype

Background: The G93A SOD1 transgenic (Tg) mouse model of ALS remains the best pathologic model for amyotrophic lateral sclerosis. The phenotype is dependent on transgene copy number, levels of mutant SOD1, genetic background, and gender. In this regard higher transgene numbers, higher levels of SOD1, SJL background, and male gender all are associated with shortened survival.

Objectives: To determine if differences in survival between male and female G93A SOD1 Tg mice on the SJL-B6 background are due to differences in the expression of human mutant SOD1(hSOD1).

Methods: All B6SJL-TgN(SOD1-G93A)1Gur mice were genotyped by quantitative PCR to verify transgene copy number. Spinal cords and brains were examined from 5 male and 5 female each of control and mutant Tg mice from four age groups: 30, 60, 90, and 120 days of age. Cerebrum and lumbar spinal cord were homogenized in either TBS (for ELISA and activity assay) or SDS lysis buffer (for Western blot) and centrifuged. Protein content of the supernatant was determined by the BCA method. Total SOD1 activity (both human and mouse) of the supernatant was determined spectrophotometrically using a kit by Oxis International. Western blots of samples were performed using antibodies to human SOD1. For quantitative purposes, each gel included three lanes loaded with 50, 100 and 200 ng of human SOD1 protein. In order to detect human SOD1, ELISAs of sample extracts (1:10000 dilution for mutant animals and 1:20 for control animals) were performed in wells coated with anti-human SOD1 antibody, then reacted with secondary antibodies conjugated with HRP.

Results: Immunoreactive hSOD1 protein undergoes a rapid upregulation in the brain and spinal cord of mutant animals at about 30 days of age that continues until about 90 days of age before leveling off. This upregulation is more pronounced in the spinal cord compared to the brain. There is no statistical difference in immunoreactive hSOD1 protein levels between males and females in the brain or spinal cord at any age. hSOD1 protein levels in control mice are undetectable. SOD1 enzymatic activity levels in mutant animals mirrored hSOD1 protein levels with a steady increase after 30 days of age with greater activity in spinal cord tissue compared to brain in both males and females. SOD1 activity levels in control animals were also greater in the spinal cord compared to the brain, slowly increasing with age but at a much slower rate; at about 8 fold lower levels than mutant animals.

Conclusion: Given that there is no difference between hSOD1 protein levels in the spinal cord of male and female Tg mice it is unlikely that variations in hSOD1 levels are responsible for the gender based differences in survival previously reported.

P30 A PREFERENTIAL LOSS OF THE LARGEST MOTOR UNITS IN FAST-TWITCH MUSCLES OF THE G93A TRANSGENIC MODEL OF ALS IS ATTENUATED BY ACTIVITY-DEPENDENT MOTOR UNIT AND MUSCLE FIBER TYPE CONVERSION

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Keywords: Functional motor units, saving motor units, model of hyperactivity

Transgenic G93A mice expressing mutant human superoxide dismutase (SOD1) with a glycine to alanine conversion at the 93rd codon develop a stereotypic syndrome with pathology and symptoms resembling human ALS (1). In both patients and G93A mice, a small proportion of motor units (MUs) remain intact in affected limbs even at end-stage disease (2,3). To explore the cellular properties that confer protection on selective motoneurones and the muscle fibres they innervate we characterised the surviving MUs throughout the lifespan of the G93A mouse. We reported an early and selective decline in the number of functional MUs in the fast-twitch tibialis anterior (TA), medial gastrocnemius (MG), and the extensor digitorum longus (EDL) muscles by 40 days of age and reduction in numbers in the slow-twitch soleus muscle (SOL) 50 days later at the onset of symptomatic disease (4). The most forceful MUs were lost first, even though the number of muscle fibres innervated by each motoneurone (innervation ratio, IR) remained unchanged. Decline in MU force, but not IR, occurred due to an increase in the proportion of smaller, less forceful type IIA and IID/X muscle fibres likely due to 1) preferential die-back of the motoneurones innervating the most forceful type IIB muscle fibres and 2) activity-dependent conversion of the remaining innervated fibres to type IIA and IID/X types. If indeed activity-dependent conversion of MUs to the more fatigue resistant type increased their resilience and hence survival, we hypothesized that experimental increase in MU activity in muscles of the G93A mouse should "save" functional MUs. To test this hypothesis, we partially denervated hindlimb muscles in G93A mice and mice expressing wild type SOD1 by avulsion of either L4 or L5 spinal roots at 40 days of age. Isometric force recordings were made from the fast-twitch TA, MG, and EDL muscles and the slow-twitch SOL muscle. Using MUNE (5) to count functional MUs at 90 days of age, we found that the rapid age-dependent decline in numbers of functional MUs in fast-twitch muscles of the G93A mice was significantly reduced by the functional hyperactivity. The muscles comprised a significantly higher component of type IIA and type IID/X fibres. We conclude that the vulnerable MUs in the G93A mouse model of ALS, can be saved by increasing their neuromuscular activity and consequently, converting them to slower, less forceful, fatigue resistant MUs.

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P31 GENE EXPRESSION IN SPINAL CORD, BLOOD, AND MUSCLE REVEALS GOOD BIOMARKERS IN BLOOD FOR ALS MOUSE MODEL

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Keywords: Biomarkers, Gene expression, mouse model

The SOD1 G93A transgenic murine model is a well-studied model for amyotrophic lateral sclerosis. Previous studies using microarray techniques have focused on spinal cord transcriptome only and revealed changes in transcription and translation, inflammation, mitochondrial preprotein translocation and respiratory chain function.

We combined transcriptional profiles of spinal cord, blood and muscle to find overlapping changes in these tissues. Information on overlapping genes will give insight into 1) common pathways in ALS and 2) the value of blood and muscle as biomarker tissue.

Whole genome gene expression profiles of spinal cord, blood and muscle from 16 transgenic mice were compared to their wild type littermates. In the spinal cord we detected a dramatic down regulation of genes involved in mitochondrial metabolism. Thirty-five percent of the significant differential expressed genes in blood of the transgenic mice were also found significant in the spinal cord. Only 0.4% of muscle significant probes were found to be significant in spinal cord. At the presymptomatic age (70 days postnatal) down regulation in mitochondrial proteins was seen in all three tissues in the transgenic mice. Up regulation of genes involved in immune response was seen in spinal cord and blood. At symptomatic age (day 100) more genes involved in immune response were up regulated in both the spinal cord and the blood.

This study provides evidence for mitochondrial dysfunction presymptomatic in spinal cord, blood which is followed by a large immune response. Overlap between changes in spinal cord and blood was large. In mice, blood could therefore serve as biomarker tissue.

P32 NEURONAL DELETION OF C-JUN IN AMYOTROPHIC LATERAL SCLEROSIS (ALS) CU/ZN SUPEROXIDE DISMUTASE 1 (SOD1) G93A TRANSGENIC MICE REDUCES NEURONAL CELL DEATH, GLIOSIS AND PROLONGS LIFESPAN

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Keywords: SOD1 G93A, c-jun, deletion

Background: Mutations in the SOD1 gene are a cause of familial ALS. Mice carrying SOD1G93A mutations develop an adult onset form of lethal motorneuron disease, implicating

programmed cell death. c-Jun is involved in neuronal survival and regeneration and its presence at increased levels in surviving spinal motoneurons of ALS patients suggests it may play a role in neurodegenerative disease.

Objectives: To investigate the effect the deletion of transcription factor c-jun has on the survival and pathology observed in transgenic SOD1G93A mice.

Methods: We crossed conditional mouse mutants lacking c-jun in the CNS (c-jun-deltaN) with transgenic SOD1G93A. Average survival times were measured using the onset of severe hind limb paralysis at the endpoint. H&E stain, Toluine-blue stains and immunohistochemistry were used to study the morphology of motoneuron, axons and glial cells.

Results: SOD1G93A; c-jun-deltaN survived significantly longer (115±3days), compared with SOD1G93A mice with c-jun (104+/24, p=5%). At day-70, before onset of ALS-like-symptoms, no morphological difference was seen between motoneuron in the L5 spinal cord segment (755±299) and axonal number in the L5 ventral root (329±250) in SOD1 G93A;c-jun-deltaN mice, compared with motoneuron (817± 230, p=58%) and axonal number (402±254, p= 40%) with SOD1 G93A mice with normal c-jun function. However, at the end stage of the disease, motoneuron (446±/251) and axonal number (253±231) were higher in SOD1 G93A; c-jun-deltaN mice, compared with motoneuron (311±/235, p,5%) and axonal number (197±215, p=16%) in control SOD1 G93A mice. In addition, both astrogliosis and microgliosis are considerably reduced in SOD1 G93A;c-jundeltaN animals compared with controls at day-70 and endstage time points.

Discussion and Conclusion: CNS-specific c-jun deletion is neuroprotective and prolongs lifespan in SOD1 G93A model of familial ALS. The SOD1 G93A; cjun-deltaN mice die with a higher number of residual motoneurons and axons, suggesting lack in adaptive mechanisms compensating for neurodegenerative loss. Furthermore, animals died despite an almost complete abrogation of microgliosis and astrocytosis suggesting that decrease in gliosis in SOD1 G93A; c-jun-deltaN mice is beneficial but not essential for their survival.

P33 LEMUR TYROSINE KINASE 2 (LMTK2) REGULATES PHOSPHORYLATION OF KLC2 AND ITS BINDING OF CARGO

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Keywords: LMTK2, KLC2, GSK3beta

Background: Defective axonal transport is one of the earliest pathological features in several transgenic mouse models of amyotrophic lateral sclerosis (ALS). Cyclin-dependent kinase 5 (cdk5) influences transport of axonal vesicles via a pathway involving phosphorylation of kinesin light chain-2 (KLC2) by glycogen synthase kinase 3beta (GSK3beta). KLC2 is a subunit of the molecular motor kinesin-1 and KLC2 phosphorylation causes detachment of kinesin-1 from vesicles, thus halting their axonal transport (1). We previously identified Lemur tyrosine kinase-2 (LMTK2) as a binding partner of the cdk5 activator p35 and showed that cdk5/p35 regulates LMTK2 activity (2). Furthermore LMTK2 binds protein phosphatase 1Calpha (PP1Calpha) and the latter is implicated in the regulation of GSK3beta. Hence, LMTK2 may be involved in the regulation of axonal transport.

Objectives: The objective of this study was to investigate the role of LMTK2 in the regulation of KCL2 phosphorylation and cargo binding.

Results and Methods: To assess if LMTK2 influences KLC2 phosphorylation, we co-expressed LMTK2, or a mutant LMTK2 incapable of binding PP1Calpha (LMTK2-deltaPP1) with KLC2 in HEK293 cells and determined KLC2 phosphorylation by 2-dimensional gel electrophoresis and Pro-Q staining. LMTK2 decreased KLC2 phosphorylation whereas LMTK2-deltaPP1 had no effect. In the same cells phospho-specific Western blots showed that LMTK2 but not LMTK2-deltaPP1 significantly increased the inhibitory phosphorylation of PP1Calpha (on Thr-320) and GSK3beta (on Ser-9). Together these results indicate that LMTK2 regulates KLC2 phosphorylation by controlling the activity of GSK3beta in a PP1Cα-dependent way. Finally, to investigate if LMTK2-induced dephosphorylation of KLC2 affected KLC2-cargo interaction we co-immunoprecipitated KLC2 and SMAD2, a specific KLC2 cargo in the presence of LMTK2. LMTK2 increased binding of KLC2 to SMAD2, showing the functional relevance of LMTK2-dependent regulation of KLC2 phosphorylation.

Conclusions: We described a novel function of LMTK2 in the regulation of KLC2-cargo interactions: LMTK2 phosphorylates PP1Calpha and hence inhibits PP1Calpha activity, which consequently causes a reduction in GSK3beta activity, and ultimately reduced KLC2 phosphorylation. Consequently, LMTK2 promotes the binding of KLC2 to cargo. LMTK2 may therefore play an important role in kinesin-1 mediated axonal transport, and thus prove a potential therapeutic target to restore normal axonal transport in ALS.

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P34 *ALS2* MRNA SPLICING VARIANTS DETECTED IN KO MICE RESCUE SEVERE MOTOR DYSFUNCTION PHENOTYPE IN *ALS2* KNOCK-DOWN ZEBRAFISH

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Keywords: ALS2, knock-out mouse, knock-down zebrafish

Background: Recessive *ALS2* mutations are linked to three related but slightly different neurodegenerative disorders: amyotrophic lateral sclerosis, hereditary spastic paraplegia and primary lateral sclerosis.

Objectives: To investigate the molecular function of alsin.

Methods: To investigate the function of the *ALS2* encoded protein, we generated *Als2* knock-out mice and z*Als2* knock-down zebrafish. A number of polyclonal antibodies specifically recognising alsin in different organisms, including human, mouse and zebrafish, have been also generated.

Results: The *Als2*-deficient mice lacking exon 2 and part of exon 3 developed mild signs of neurodegeneration compatible with axonal transport deficiency. In contrast, z*Als2*

knock-down zebrafish had severe developmental abnormalities, swimming deficits and motor neuron perturbation. We identified, by RT-PCR, Northern and Western blotting novel *Als2* transcripts in mouse central nervous system. These *Als2* transcripts were present in *Als2* null mice as well as in wild type littermates and some rescued the zebrafish phenotype.

Discussion: Thus, we speculate that the newly identified *Als2* mRNA species prevent the *Als2*-KO mice from developing severe neurodegenerative disease and might also regulate the severity of the motor neurons phenotype observed in ALS2 patients.

P35 MITOCHONDRIAL ALTERATIONS IN TRANSGENIC MICE WITH AN H46R MUTANT SOD1 GENE

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Keywords: mitochondria, ultrastructure, H46R mutant SOD1 transgenic mice

Background: H46R mutant Cu/Zn superoxide dismutase (SOD1) transgenic (Tg) mice are a model for human familial ALS, characterized by initial muscle weakness and atrophy in the legs, a very long-term clinical course (approximately 15 years) and pathologically, widespread changes of the spinal cord that extend beyond the motor system, including many aggregates lacking vacuoles. No report on morphological changes of mitochondria has yet been published in these transgenic mice.

Objectives: To examine ultrastructural changes of mitochondria in H46R mutant SOD1 mice, and to clarify whether the mitochondrial alterations affect the pathomechanism of the Tg mice.

Methods: We electron-microscopically examined the spinal cord of Tg mice expressing an H46R mutant human SOD1 gene at early pre-symptomatic (12 w, n = 2), late presymptomatic (16 w, n = 2), early symptomatic (20 w, n = 2), and end (24 w, n = 2) stages, respectively. Age-matched non-Tg mice served as controls in each group. We also studied immunoelectron-microscopically the spinal cord of Tg mice at late presymptomatic (n = 1), early symptomatic (n = 1), and end (n = 1) stages, respectively, using post-embedding method.

Results: Non-Tg littermates: Small vacuolar changes and disorganization of the inner compartment of mitochondria were only occasionally seen.

Tg mice: In 12-week-old mice, largely swollen mitochondria with an increased number of cristae and mitochondria with disorganized inner compartment were only occasionally observed predominantly in the axon of the anterior column and, to a lesser degree, dendrites and somata of anterior horn neurons. In 16-week-old mice, in addition to those observed in 12-week-old mice, the alterations of the cristae such as electron-dense changes, multi-folded membranous structures and electron-dense amorphous membranous structures were only occasionally observed in proximal axons. In 20-week-old mice, alterations of the cristae observed in 16-week-old mice were more frequently observed in the proximal axons. Inner and outer membranes focally projected outward slightly on both the transverse and longitudinal sections. In 24-week-old mice, above-described alterations of the cristae became prominent and were frequently observed not only in the proximal axons, but also in the somata, dendrites, and presynaptic terminals. The whole interior of mitochondria frequently changed into electron-dense membranous or amorphous structure. Electron-dense membranous structures of inner and outer membranes prominently projected outward. Immunoelectron-microscopically, neither normal-appearing nor degenerated mitochondria exhibited SOD1- or ubiquitin-immunogold labeling.

Conclusions: Mitochondrial alterations may be involved in the pathomechanism of motor neuron degeneration in H46R mutant SOD1 Tg mice, probably by disrupting axonal transport of substrates needed for neuronal viability and thus causing motor neuron death.

P36 SLOW DEATH OF MOTOR NEURONS IN SPORADIC ALS MOUSE MODEL BY CONDITIONAL TARGETING OF RNA EDITING ENZYME ADAR2

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Keywords: ADAR2, RNA editing, knockout mouse

Background: Deficient RNA editing of GluR2 mRNA at the Q/R site occurs specifically in motor neurons of sporadic ALS patients regardless of disease phenotype; i.e. classical ALS, progressive bulbar palsy and ALS-dementia. On the contrary, this molecular change does not occur in dying motor neurons of other motor neuron diseases including familial ALS associated with mutated SOD1 (ALS1). Because adenosine deaminase acting on RNA type 2 (ADAR2) specifically catalyzes GluR2 Q/R site-editing, it is likely that ADAR2 activity is not sufficient to edit this site in motor neurons of sporadic ALS. Because these molecular changes occur in a disease and motor neuron-specific manner, we have proposed that GluR2 Q/R site-underediting due to ADAR2 underactivity is a neuronal death-inducing cause in sporadic ALS. Systemically ADAR2 knockout mice die young from status epilepticus, suggesting the role of ADAR2-mediated RNA editing in neuronal survival.

Objectives: To investigate whether deficient ADAR2 activity induces slow neuronal death as seen in motor neurons of sporadic ALS.

Methods: We have generated genetically modified mice in that the ADAR2 gene is conditionally knocked out in motor neurons using Cre-loxP system. After confirming that ADAR2 was deficient in a subset of motor neurons, we investigated the characteristics of phenotype of the mutant mice by means of weekly measurement of stay time on Rotarod and grip strength, analysis of survival time, and of histological observation of central nervous system.

Results: The mutant mice displayed significantly shorter mean survival than control mice and progressive motor-selective deficit in behaviour with delayed loss of spinal motor neurons. RNA editing at the GluR2 Q/R site was completely abolished in ADAR2-lacking motor neurons. The extent of editing at the CYFIP2 K/E site (a recently identified A-to-I editing position) was markedly decreased in motor neurons deficient for ADAR2 and GluR2 Q/R site-editing.

Discussion and Conclusions: The mutant mice exhibited an ALS-like phenotype without developing fatal epilepsy. Our results indicate that motor neurons would undergo slow progressive death if ADAR2 were deficient. Thus it is likely that a reduction of GluR2 Q/R site-editing in motor neurons of sporadic ALS is caused by ADAR2-underactivity and a direct cause of neuronal death. Our model would be useful in searching the aetiology and therapy of sporadic ALS.

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P37 CHANGES OF NOGO-A AND RECEPTOR NGR IN THE LUMBAR SPINAL CORD OF ALS MODEL MICE

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Keywords: Nogo-A, NgR, spinal cord

Background: Nogo-A is a myelin-associated neuronal growth inhibitor protein, and has been identified as the target of a neutralizing antibody for a myelin protein. Nogo-A delivers it's inhibitory signals through it's receptor, Nogo receptor (NgR). High expressions of Nogo-A and NgR expression patterns were reported in trauma, stroke, and some neurodegenerative diseases; Nogo-A and NgR have been considered as possible therapeutic targets. Changes in the expression pattern of Nogo-A have also been reported in ALS (1, 2).

Objectives: Detailed assessment of Nogo-A and its receptor NgR at the spinal cord of the ALS mouse models or patients has not been reported. Therefore, we examined the expression and distribution pattern of Nogo-A and NgR in an ALS mouse model to determine whether this molecule plays a role in this disease.

Methods: We used 10, 15, and 18 weeks-old (W) G93A (Tg) mice and age-matched non-Tg wild-type littermates (WT) as a control. Ten W Tg mice were considered as the pre-symptomatic stage, 15 W as early-symptomatic stage, and 18 W as end stage of the disease. After dissecting lumbar spinal cord from each mouse, we performed Western Blot and immunohistochemistry analysis.

Results: As compared with WT mice, Tg mice showed that the expression levels of Nogo-A transiently increased in motor neurons at an age of 10 W, while it progressively decreased from 15 to 18 W. NgR expression in motor neurons of the Tg mice increased at 10 W, then progressively decreased from 15 to 18 W. In contrast, there was no significant change in the dorsal lumbar cord or the cerebellum of Tg mice throughout the progression of ALS.

Discussion and Conclusions: This study suggests that the function of Nogo-A may alter under certain conditions and locations, and thus transient overexpression of Nogo-A and NgR in motor neurons of this ALS mouse model at 10 W may represent a survival reaction of these cells under stressful conditions. These proteins then gradually decrease with disease progression probably due to depletion of energy in motor neurons, where mitochondrial disfunction was reported (3).

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P38 EXPRESSION OF NOGO IN THE SPINAL CORD AND MUSCLE DURING DISEASE PROGRESSION IN THE G93A SOD1 MOUSE MODEL OF ALS/MND

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Keywords: NOGO, Spinal cord, muscle

Background: Compensatory synaptic re-organisation in motor pathways in ALS patients and murine models (1), may be restricted by release of neurite outgrowth inhibitors like NOGO. Raised NOGO expression has been reported in muscle homogenates from autopsied ALS patients and end-stage SOD1 mice (2). Irrespective of whether this is disease specific (3), NOGO limits neurite outgrowth in the injured spinal cord (4), but it's expression in the spinal cord in ALS has not been reported, and it's time-course of expression in disease development remains uncertain.

Objective: To explore NOGO in spinal cords and muscles of $SOD1^{G93A}$ mice during disease progression.

Methods: Transgenic (Tg) $SOD1^{G93A}$ mice, and wild-type littermates (WT) were examined at pre-symptomatic (6 &13 wks) and symptomatic stage (18 wks). Cryostat sections of spinal cords were immunostained for NOGO-A (MAb clone 6D5, GSK 2), and tibialis anterior (TA) muscles were immunostained for NOGO-A (Abcam), NOGO-B & NOGO–C (Chemicon). SABC methods detected tissue-bound antigen. Image analysis measured cell size and staining levels under standardised conditions. Immunoblots confirmed both NOGO-A antibodies bind to NOGO-receptor NgR1(protein from R&D Systems), and validated immunostaining. Histochemistry typed muscle fibres.

Results: *a) Spinal cord;* NOGO-A localised to MN and oligodendrocytes (OD) in both Tg and WT at all ages. Relative to age-matched WT mice, MN in Tg mice showed raised levels of NOGO by 13 wks ($p < 0.03$), but lower levels at 18 wks reflecting profound MN damage. Significantly higher numbers of NOGO-A immunostained OD occurred in the anterior horn and ventral white matter than in other regions of the cord in Tg mice. Increases first occurred at 13 wks ($p < 0.01$), progressing further in symptomatic mice ($p < 0.007$). *b) TA muscle;* In WT mice of all ages, superficial regions of the TA contained mostly larger fibres with 'low' NOGO immunostaining. Their size and number resembled the histochemical profile of Type 2B fibres which dominate the superficial TA. Deeper regions contained smaller fibres with 'high' NOGO immunostaining, resembling Type 2A fibres. This pattern occurred for all NOGO-isoforms. Relative to WT mice, *i)* Tg mice at 6 wks, showed no differences in the regional pattern of immunostaining. Larger unstained fibres dominated superficial regions, with higher numbers of stained fibres in deeper regions in all isoforms. *ii)* By 13 wks, Tg mice showed mild fibre atrophy with significant increases in staining in the superficial TA for all isoforms ($p < 0.0001$), but variable changes in deeper regions (NOGO-A & -C, $p > 0.1$ to $p < 0.001$; NOGO-B, $p < 0.03$) *iii)* In symptomatic mice (18 wks), the major alteration was to the superficial region which showed severe muscle fibre atrophy

($p < 0.0001$), and significantly increased staining for NOGO-A, B & C ($p < 0.0001$).

Conclusions: NOGO up-regulation begins presymptomatically in spinal cord and muscle, and progresses until end-stage. In the spinal cord, the response targets the ventral motor areas. In the TA. the response shows preferential targeting of Type 2B containing regions, implying impaired capacity for reinnervation. On-going work using strategies targeting NOGO aim to improve compensatory reinnervation.

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P39 IDENTIFICATION OF AXON-MEDIATED NEURONAL SIGNALING TO ASTROCYTES. THE ENDOGENEOUS REGULATION ASTROGLIAL SPECIFIC SYNAPTIC PROTEINS IS DISRUPTED IN ALS

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Keywords: astroglia, synapse, glt1

Background: Astrocytes play an essential role in the regulation of synaptic function and neuronal metabolism. The communication for this regulation between neuron/axon and astroglial remains unknown. In disease settings, severe disruption of this neuron-glial network, as reflected by a loss of synaptic astroglial glutamate transporter EAAT2/GLT1 and MCT1 has been repeatedly documented. Disruption of this astroglial function is a potently toxic mechanism that acts to promote disease progression in neurodegeneration associated with amyotrophic lateral sclerosis.

Objectives: To investigate various *in vitro* and *in vivo* models of the up- and down-stream signaling from neuron to astrocytes that regulate GLT1 expression.

Methods: We first developed a novel neuron astrocyte co-culture system by using a microfluidic platform that only allows axons to approximate and/or contact individual astroglia. By using this novel system, we showed that axons are sufficient and necessary to induce GLT1 expression through transcriptional activation in astrocytes, by both membrane contact and secretion of soluble factors. Axon mediated transcriptional activation of GLT1 in astrocytes is dependent on synaptic transmission from neuron to astrocyte as tetrodotoxin (TTX) and antagonists of both ionotropic and metabotropic glutamate receptors block neuronal induction of GLT1 in slice and astrocyte cultures. Furthermore, *in vivo* denervation of neuron to astrocyte signaling by acutely or chronically inducing neuron degeneration with Ricin or over-expression of mutant SOD1 also reduces both the GLT1 genomic promoter activity and GLT1 mRNA levels in lumbar spinal cord astrocytes. Subsequent computational and mutagenesis analysis of EAAT2/GLT1 promoter further identified a *cis*-element that is essential to the EAAT2/GLT1 promoter activity. By affinity purification and LC/MS, a kappa B-motif binding phosphoprotein was identified that specifically binds to this essential *cis*-element. The expression of kappa B-motif binding phosphoprotein is highly correlated to the expression of GLT1 in astrocytes under different physiological and pathological conditions. Moreover, silencing of kappa B-motif binding phosphoprotein results in reduced GLT1 expression in cultured astrocytes and *in vivo*, suggesting that kappa B-motif binding phosphoprotein is essential for GLT1 activation.

Conclusions: Taken together, we demonstrated that synaptic transmission from neuron to astrocyte is sufficient and essential for astroglial synaptic protein GLT1 induction. Axonal injury /dysfunction associated with diseases alters a key astroglial transcriptional regulatory pathway, kappa B-motif binding phosphoprotein and regulation of this essential synaptic protein EAAT2/GLT1, ultimately contributes to further neurodegeneration.

P40 ALS CAUSES ALTERED PROLIFERATION AND CYTOPLASMIC INJURY TO NG+ CELLS IN ADULT SPINAL CORD

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Keywords: NG2, stem cell, differentiation

Background: Amyotrophic lateral sclerosis (ALS) is characterized by the specific loss of motor neurons in the lumbar spinal cord as well as focal abnormalities in astroglia. Evidence from human patients as well as from the animal model for ALS suggests that non-neuronal cells significantly contribute to the death of motor neurons and the progression of disease. $NG2^+$ cells are prevalent in the adult CNS and are thought to represent a population of progenitor cells capable of generating new oligodendrocytes and astrocytes in the injured adult CNS. Additionally, $NG2^+$ cells have been shown to express neurotransmitter receptors that are activated at neuro-glia synapses.

Objectives: To study the biology of endogenous NG2 population in rodent models and human ALS and to follow the differentiation and reaction of these cells in ALS and what role they play in generation of new astroglia.

Methods: We employed NG2 BAC promoter DsRed reporter mice to study the developmental population of endogenous MG2 cells in wild type and ALS mice in multiple CNS regions and time points. We examined the differentiation of the cells into new astroglia and oligodendroglia in ALS mice. In addition we examined the population of NG2 cells in human ALS and non-ALS spinal cord.

Conclusions: We have found that there are significantly more $NG2^+$ cells in the spinal cord of diseased mSOD1 mice compared to pre-symptomatic mice. Additionally, we have found that $NG2^+$ cells in the diseased spinal cord display signs of pathologic injury. Most importantly we show a significant population of new astrocytes in disease spinal cord are derived from differentiating NG2 adult progenitors. These findings suggest that $NG2^+$ cells not only respond to mSOD1-mediated damage, but may also become diseased. Furthermore, we are studying how the normal activity of these cells is altered in the disease setting. These studies offer tantalizing information regarding the function of this distinct class of glial cells, as well as the ability of the adult CNS to generate new oligodendrocytes and astrocytes in disease.

P41 COMPLEMENT THERAPY PROMOTES PERIPHERAL NERVE REGENERATION

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Keywords: regeneration, complement, animalmodel

Background: The complement system (C), highly expressed in the healthy peripheral nerve, is activated during Wallerian degeneration (WD). Using a C6 deficient rat strain that is unable to form the membrane attack complex ((MAC, C5b-9), the terminal activation product of the C cascade) we showed that MAC is required for rapid WD after peripheral nerve injury. Pharmacological inhibition of C activation with soluble C receptor 1 (sCR1) protected the nerve from axonal and myelin breakdown at 3 days post-injury; it inhibited macrophage infiltration by five fold and prevented their activation.

Objectives: To determine the effect of C inhibition on nerve regeneration after acute trauma.

Methods: We performed a crush injury of the sciatic nerve in C6 deficient rats and rats treated systemically for 1 week with sCR1. Recovery of function was assessed by footflick test every week up to 5 weeks post-injury. Axonal regeneration was monitored by retrograde tracing of sensory neurons at 1 week post-injury and pathology of the tibial nerve at 5 weeks post-injury.

Results: Both, genetic and pharmacological inhibition of C activation resulted in faster recovery of sensory function than PBS-treated animals whereas reconstitution with purified human C6 protein re-established the wildtype phenotype. The number of sensory neurons that could be labeled by retrograde tracing in C6 deficient rats was significantly higher than wildtypes. Neuropathological analysis of the sciatic nerve after 5 weeks recovery showed that C6 deficiency and sCR1 treatment improve regeneration as judged by axon diameter, myelin thickness and number of regenerative clusters.

Conclusion: We conclude that complement inhibition improves regeneration of the injured peripheral nerve.

Discussion: We propose that an early and destructive complement-mediated event during WD hampers the subsequent regenerative process. Interfering with the terminal C cascade offers an interesting therapeutic approach for many injury and non-injury related disorders in which activation of the C system has been implicated.

Amyotrophic lateral sclerosis (ALS) is an example of such diseases. We observed several components of the complement cascade, including C1q, C3c and C3d in active microglia and reactive astrocytes in spinal cord and motor cortex of both sporadic and familial ALS cases.

P42 ENHANCED NEUROMUSCULAR SYNAPTIC PROTECTION INDUCED BY MODIFIERS OF THE NEUROPROTECTIVE WLDS GENE

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Keywords: Neuromuscular junction, neuroprotection, WldS mouse

Background: Loss of neuromuscular junctions (NMJ's) precedes other signs of neurodegeneration in SOD1-dependent familial ALS. More generally, progressive muscle denervation with or without compensatory innervation by collateral sprouts from relatively healthy neurones is a feature of sporadic as well as familial ALS. Reducing the rate of neuromuscular synaptic loss and/or strengthening compensatory sprouts could therefore significantly preserve muscle utility, function and strength in ALS patients and thus both mitigate progression of disease and improve quality of life.

Objective: To seek generic mechanisms that protect motor nerve terminals from degeneration.

Methods: The principal model we used was the WldS mutant mouse, in which axotomy produces about a 10 fold delay of Wallerian degeneration after nerve injury. Axons are strongly protected by WldS protein expression but protection of neuromuscular synapses is much weaker; highly sensitive to the WldS gene dose; and declines progressively with age in native mutant WldS mice. We have adopted three basic strategies for identifying enhancers of neuromuscular synaptic protection using this mutation: first, systematic mutation of defined regions of interest in the WldS gene itself; second, random genomic point mutagenesis, using sensitized, heterozygous WldS as a background, with high-throughput screening for relevant progeny; and third, 'environmental' influences that alter neuromuscular activity. We assess the efficacy of the first strategy in transgenic lines using standard physiological and immunocytochemical techniques. For the second strategy, we screen progeny in anaesthetized F1 mice using confocal fibre-optic microendoscopy, 3 days after cutting the sciatic nerve. Candidate modifiers (phenodeviants) are recovered and used for inheritance testing of the neuromuscular protective phenotype. Thirdly, we either block activity chronically in peripheral nerves using tetrodotoxin infused from implanted microcapillaries; or enhance activity by giving mice access to exercise (running) wheels. Outcome is measured as in the first strategy.

Results: All three approaches have yielded evidence of modulated neuromuscular synaptic protection compared with native mutant WldS mice. From the first approach, we have found that targeted mutations in the chimeric WldS gene, followed by transgenic expression in mice, leads to significantly greater protection of NMJ's 3 days after axotomy compared with heterozygous WldS mice. In the second

approach, we have identified a candidate ethylnitrosourea (ENU)-induced mutant in which there is also enhanced protection of some motor nerve terminals after axotomy. The inheritance of this phenotype is currently under test. From the third approach, preliminary data suggest that neuromuscular activity may condition the vulnerability of motor nerve terminals to axotomy-induced degeneration.

Conclusions: The data obtained thus far support a model in which neuromuscular synapses are an independent neurodegenerative compartment. Further study will include tests of the effects of the modifiers we have identified on SOD1 mouse models of ALS. This may help identify treatments that protect NMJ's from degeneration more effectively than WldS, an important step towards the goal of mitigating disease progression in either familial or sporadic ALS.

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P43 LOSS OF FUNCTION OF *FIG4* CAUSES AN ASYMMETRIC NEURONAL DEGENERATION WITH RAPID PROGRESSION

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Keywords: Fig4 gene, progression of neurodegeneration, intracellular organelle trafficking

Background: We have recently identified a novel recessive disorder Charcot-Marie-Tooth type-4J (CMT4J) in humans and in pale tremor mice *(plt)* that is caused by mutations in the *Fig4* gene that encodes a $PI3,5P_2$. These homozygous mutant mice develop neurodegeneration with intracellular vacuoles in both the central and peripheral nervous system. However, a detailed analysis of the natural history of CMT4J remains to be described, and the mechanisms responsible for the effects of *Fig4* mutations have not been identified.

Objectives: The present study was to investigate clinical, pathological, and electrophysiological abnormalities in CMT4J patients and in mice with loss of function of *Fig4*.

Methods: Two siblings are compound heterozygotes for mutations of *Fig4*, and were evaluated with serial neurological examinations, electrophysiological, pathological studies and time-lapse imaging. Eleven mice at six weeks of age and two mice at 3 weeks of age were also examined with similar techniques.

Results: We describe the 9-year clinical course of CMT4J, including asymmetric, rapidly progressive paralysis, in two siblings. Progression in both was preceded by trauma. Sensory symptoms were absent despite reduced numbers of sensory axons. Thus, the phenotypic presentation of CMT4J clinically resembles motor neuron disease. Time-lapse imaging of fibroblasts from CMT4J patients demonstrates impaired trafficking of intracellular organelles because of obstruction by vacuoles. Further characterization of *plt* mice identified axonal degeneration in motor and sensory neurons, limited segmental demyelination, lack of TUNEL staining and lack of accumulation of ubiquitinated protein in neuronal vacuoles.

Discussion and Conclusion: This study represents the first documentation of the natural history of CMT4J. The progression pattern of this disease suggests that CMT4J and *plt* mice may be a unique model for investigating the progression of motor neuron degeneration. Physical obstruction of organelle trafficking by vacuoles is a potential novel cellular mechanism of neurodegeneration.

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THEME 3 IN VITRO EXPERIMENTAL MODELS

P44 SIGNALS FROM CNS EXTRACTS OF SOD1-G93A MICE PROMOTE hMSCS NEUROGENIC DIFFERENTIATION IN VITRO

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Keywords: SOD1-G93A mice, CNS extracts, neurogenic differentiation

Background: Amyotrophic lateral sclerosis (ALS) is a progressive, lethal, neurodegenerative disease, currently without any effective therapy. Multiple advantages make mesenchymal stromal cells (MSC) a good candidate for cellular therapy of ALS, but the capabilities of MSCs to differentiate into neurons in ALS mice still need to be proven.

Objectives: To investigate whether signals from pathological CNS extracts of SOD1-G93A (20 weeks of age) ALS mice promote hMSCs neurogenic differentiation in vitro.

Methods: The brain and spinal cord of SOD1-G93A mice at symptomatic stages (20 weeks of age) and normal mice were removed and homogenized by adding DEME, respectively. The supernatant was collected and filtered, then turned into a conditioned medium for the hMSCs. Passage 5 of hMSCs was seeded into a 6-well plate and cultured with the conditioned medium. Proliferaton of hMSCs was observed by phase-contrast light microscope. The cell viability and the phenotype of hMSCs and neural like cells were analyzed by FMC, RT-PCR, immunofluorescence, and Western blots, respectively.

Results: Incubation of hMSCs with the conditioned medium prepared from the CNS extracts of SOD1-G93A mice resulted in a time-dependent morphological change from fibroblast-like into neuron-like. Similarly, the expression of Nestin and subsequent beta-tubulin III, NSE and GAP43 increased over time. Moreover, signals in the pathological CNS extracts of SOD1-G93A mice were more effective in promoting hMSCs neurogenic differentiation than those in physiological extracts of normal adult mice.

Conclusion: These results show that the pathological condition of ALS is endowed with capacity to induce hMSCs neurogenic differentiation and that the hMSCs may be a potential candidate in cellular therapy for ALS.

P45 PREVENTION OF EXCITOTOXIC MOTONEURON DEATH BY LITHIUM IN ORGANOTYPIC SLICE CULTURES OF SPINAL CORD

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Keywords: lithium, excitotoxicity, motoneuron

Background: Several *in vitro* and *in vivo* studies have reported the neuroprotective effects of lithium (Li) and suggest its potential in the treatment of acute and chronic neurological disorders. Recently, it has been demonstrated that Li delays the progression of amyotrophic lateral sclerosis (ALS) in animal models and humans (1). We have recently developed an organotypic slice culture of chick embryo spinal cord that provides a convenient model for *in vitro* studies on mature motoneurons (MNs) (2).

Objectives: Since we have found that chick MNs in organotypic spinal cord cultures are vulnerable to excitotoxic insults, the presumptive neuroprotective effects of Li in this paradigm has been investigated.

Methods: Slices of lumbar spinal cord from E16 chick embryos were cultured for 7 days *in vitro* (DIV) and treated with different regimens of kainate (KA) in the absence or presence of LiCl (4–10 mM) and chronically applied. MN survival and ultrastructural morphology was evaluated and the neuroprotective mechanisms of Li on this system were analyzed. Moreover, LiCl was chronically administered *in ovo* in order to evaluate its effects on programmed cell death of MNs during development.

Results: LiCl inhibited excitotoxic MN death induced by KA in a dose dependent manner. However, MNs rescued by Li displayed conspicuous structural changes consisting of an accumulation of neurofilaments, increased large dense core vesicles and autophagic vacuoles. In congruence with these changes, an increased immunostaining was found for: phosphorylated neurofilaments, calcitonin gene-related peptide (CGRP) and LC3. Li treatment resulted in an inhibition of GSK-3β and indirubin-3'-monoxamine, a specific GSK-3β inhibitor, mimicked the effects of Li. LY294002, a PI3-K inhibitor, blocked the anti-excitotoxic effects of Li. The involvement of the inositol pathway in the neuroprotective effects of Li was also explored with negative results. Li was not able to prevent programmed (apoptotic) MN death when administered *in vivo*.

Conclusions: In an *in vitro* paradigm, Li protects mature MNs against excitotoxic injury induced by KA. These effects appeared to be mediated by the inhibition of GSK-3β and PI3-K/Akt pathways. Li-rescued MNs remains alive but exhibited prominent structural abnormalities indicative of chronic pathology. In addition, Li does not prevent developmentally regulated and target dependent apoptotic MN death in the chick embryo.

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P46 HUMAN NMDA RECEPTORS MODULATION BY SERA FROM AMYOTROPHIC LATERAL SCLEROSIS PATIENTS AND MUTATED SUPEROXIDE DISMUTASE TRANSGENIC RATS

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Keywords: excitotoxicity, NMDA, sera

Amyotrophic lateral sclerosis (ALS) is a devastating neuromuscular disease, characterized by the selective degeneration of the superior motor neurons in the motor cortex and of the inferior motor neurons in the brain-stem and spinal cord. So far, no treatment has achieved significant improvement. The familial form of the illness is associated with the mutation of the superoxide dismutase enzyme (SOD-1), but it accounts for fewer than 10% of cases; the rest, more than 90%, correspond to the sporadic form.

In this study we tested the effect of sera from sporadic ALS patients and from mutated human SOD-1 (mSOD1 G93A) transgenic rats on N-methyl-D-aspartate receptors (NMDAR). We hypothesize that an endogenous excitotoxic factor is implicated in neuronal death in ALS, mediated by the activation of NMDAR noncanonical signalling pathways (1).

Sera from ALS patients or healthy subjects were pretreated to inactivate complement pathways and dialysed to remove glutamate. Sera from mSOD1 G93A rats were obtained at different stages of the neurodegenerative progression. Sera from transgenic rats were also pretreated to eliminate complement system and glutamate. Human NMDAR were expressed in *Xenopus laevis* oocytes, and glutamate-induced currents were recorded using the two electrode voltage clamp technique

We observed that sera from sporadic ALS patients induced transient oscillatory currents in *Xenopus* oocytes expressing NMDAR with a total electric charge (14594 ± 603 nC, $n = 76$ oocytes) significantly higher than the electric charge carried by currents induced by sera from healthy subjects (4219 ± 206 nC, $n = 76$ oocytes) $p < 0.001$. The currents were inhibited by MK-801, a noncompetitive blocker of NMDAR. Results of sera from mSOD1 G93A transgenic rats were similar to those of sera from ALS patients; samples from patients with another type of neuromuscular disease did not exert this effect.

The oscillatory currents recorded are due to internal calcium mobilization (2), our data therefore agree with the view that ALS patients sera contain some soluble factor/s that activates intracellular calcium concentration.

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P47 THE INVOLVEMENT OF THE KYNURENINE PATHWAY AND INFLAMMATION IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: kynurenine pathway, quinolinic acid, NSC-34

Background: Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron degenerative disease that selectively targets motor neurons in the motor cortex, brain stem and spinal cord. The kynurenine pathway (KP), which catabolizes tryptophan and generates neuroactive compounds such as picolinic acid (PIC) and quinolinic acid (QUIN), is emerging as a possible pathogenic component of ALS. The first enzyme in the KP, indoleamine-2,3 dioxygenase (IDO), can be stimulated by cytokines.

Objectives: This study aims to characterize the KP in ALS patients and the NSC-34 rodent motor neuron cell line; and assess the effect of QUIN toxicity on NSC-34 cells.

Methods: GC/MS and HPLC were used to analyze CSF and serum QUIN and PIC levels and IDO activity, respectively, of ALS patients ($n = 155$) and controls ($n = 20$). Antibodies to HLA-DR, IDO and QUIN were used on paraffin embedded ALS human spinal cord and motor cortex sections. In NSC34 cells, RT-PCR and immunocytochemistry were used to characterize KP enzymes and catabolites. LDH test assessed the effect of QUIN, with and without antagonist MK801, APV and memantine.

Results: IDO activity was significantly increased in ALS patients and ALS serum and CSF PIC levels were significantly lower but serum QUIN levels were significantly higher. ALS motor cortex and corticospinal tracts showed extensive microglial activation. IDO immunoreactivity was evident in the ventral horn motor neurons and in the motor cortex neurons, including remaining Betz cells. QUIN immunoreactivity was seen mostly in glial cells and in motor cortex and spinal cord neurons. NSC34 cells stained positive for KP enzymes and catabolites and RT-PCR showed the presence of most of the KP enzymes. LDH production displayed a dose dependant increase with QUIN which was partially inhibited by MK801 and completely inhibited by MK801, APV and memantine combined.

Conclusion: Our results provide *in vivo* and *in vitro* support for the involvement of KP in ALS.

P48 A NOVEL MOTONEURON DEATH PATHWAY MEDIATED BY THE TNF SUPERFAMILY LIGAND, LIGHT

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Keywords: motoneuron death signalling, LIGHT, caspase

Death pathways restricted to specific neuronal classes could explain the selectivity of neuronal loss in neurodegenerative diseases, such as the loss of motoneurons in amyotrophic lateral sclerosis (ALS). We previously showed that Fas-induced death of motoneurons involves a motoneuron-specific cell death pathway, which is exacerbated in motoneurons

expressing ALS-linked mutated SOD1 (1,2). Importantly, involvement of the Fas death pathway in the pathogenesis has been documented (3). However, the Fas death pathway may not be responsible for the loss of all motoneurons, suggesting that other death pathways might be implicated.

LIGHT is a member of the tumor necrosis factor receptor (TNFR) superfamily, which upon binding to the herpes virus entry mediator (HVEM) and/or the lymphotoxin-β receptor (LT-βR) can trigger the death program. In this study we show that cultured motoneurons express both LT-βR and HVEM, and that soluble recombinant LIGHT induces death of approximately 50% of motoneurons in a dose-dependent manner. LIGHT-mediated death might be motoneuron-specific since striatal, cortical, hippocampal and sensory neurons are resistant to LIGHT effect. We show that LIGHT is additive to FasL since both ligands together induce death of about 70% of cultured motoneuron. To our surprise, LIGHT-induced motoneuron death is triggered in a Caspase-9 and -6 dependent but Caspase-8 and -3 independent manner.

This study indicates that LIGHT acts as a selective trigger of motoneuron death *in vitro,* which involves an unconventional caspase pathway. We are currently investigating the potential involvement of LIGHT death signalling in the pathogenesis of motoneuron disease.

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P49 DO ALS ASSOCIATED SOD1 MUTANTS INTERACT WITH LIPID MEMBRANES?

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Keywords: SOD1, membranes, stability

More than 130 mutations in the human gene encoding the cytosolic homodimeric enzyme Cu/Zn-Superoxide Dismutase (SOD1) have been linked to the familial form of ALS (fALS). The key event in SOD1 associated fALS seems to be the pathological formation of toxic protein aggregates formed by initially unfolded or partly structured SOD1 protein mutants.

We have compared the folding behavior for a set of SOD1 mutants. The common denominator for all of them is the move of the folding equilibrium towards a denatured monomer. It is believed that these denatured monomers gain properties which might be toxic to the cell. The apo form of the protein is partly folded under reduced conditions and therefore exposes both a hydrophobic dimer-interface but charged patches. This exposure is ideal for interactions with cellular membranes to occur.

Our results show electrostatic interactions between the reduced apo form of ALS associated SOD1-mutants and charged membrane surfaces. The interaction changes the secondary structures of these mutants in a way that is quite different from the situation found in the membrane free aqueous environment. Mutants of SOD1 seem to interact differently compared to wildtype SOD1.

P50 CU,ZN SUPEROXIDE DISMUTASE IS SECRETED VIA EXOSOMES IN A CELL MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: SOD1, exosomes, NSC-34 cells

Background: A familial form of the neurodegenerative disease amyotrophic lateral sclerosis (ALS), is caused by dominant mutations in the cytosolic Cu,Zn superoxide dismutase (SOD1). There has been evidence for secretion of SOD1, by an unknown mechanism that might involve a vesicle dependent pathway. SOD1 is a cytoplasmic protein that lacks a signal peptide, so there must be an alternative mechanism for the extracellular export of this protein.

Exosomes are small membrane vesicles (30–100 nm diameter) secreted by various cell types, including neurons as a consequence of fusion of multivesicular late endosomes/lysosomes with the plasma membrane.

Objectives: To investigate the capacity of NSC-34 cells expressing wild-type or mutant hSOD1 to secrete SOD1 protein via exosomes.

Methods: Stable mouse motor neuron-like NSC-34 cells overexpressing human SOD1 wild-type $hSOD1^{wt}$ (NSC-34/$hSOD1^{wt}$) and mutant $hSOD1^{G93A}$ (NSC-34/$hSOD1^{G93A}$) have been used as an ALS cellular model.

Results: Wild-type and mutant SOD1 were present in the supernatant medium from NSC-34 cells, and they were found to be secreted in association with a membrane fraction, vesicle tethering, was containing a mixture of exosomes and apoptotic membrane blebs, that pelleted at 100,000 $\times g$. Sucrose density gradient separation of this fraction showed that wild-type and mutant SOD1 were found between 0.5 and 1.16M sucrose and co-localized with the exosomal marker CD9. Therefore, SOD1 secretion occurred via exosomes. p115, a cytosolic and Golgi apparatus protein involved in vesicle tethering was also found in exosomes, contrary to the endoplasmic reticulum protein calnexin.

Discussion and Conclusions: SOD1 secretion mediated by exosomes might constitute a new cellular mechanism of cell-to-cell communication and dissemination of mutant SOD1 toxicity in ALS pathogenesis.

P51 AAV-MEDIATED EXPRESSION OF WILDTYPE OR MUTANT VAP-B TRIGGERS DEATH OF EMBRYONIC MOTONEURONS THROUGH IMPAIRMENT OF ENDOPLASMIC RETICULUM FUNCTIONS

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Keywords: ALS, VAP-B, ER stress

Amyotrophic Lateral Sclerosis (ALS) is a late-onset motoneuron disorder characterized by selective loss of motoneurons in the spinal cord, brainstem and motor cortex. Disturbed

homeostasis of the early secretory pathway is a pathological hallmark of the disease. However, molecular mechanisms leading to motoneuron death following impairment of the endoplasmic reticulum (ER) function remain unclear.

Recently, an autonomic dominant mutation (P56S) in VAMP-associated protein subtype B (VAP-B) has been associated with familial forms of ALS. VAP-B, an endoplasmic reticulum (ER) resident protein and ER-Golgi intermediate vesicle, has been proposed to play a role in ER-Golgi transport and in the unfolded protein response (UPR). A P56S mutation leads VAP-B to form cytosolic aggregates that might impair the early secretory pathway. It remains unknown how VAP-B^{P56S} leads to motoneuron degeneration.

Adeno-Associated Viruses (AAV), which can transduce up to 90% of purified motoneurons, were used to express either wild type ($hVAPB^{wt}$) or mutant forms ($hVAP\text{-}B^{P56S}$) of human VAP-B. We show that $hVAP\text{-}B^{WT}$ localizes to the ER and ER-Golgi intermediates, whereas $hVAP\text{-}B^{P56S}$ forms cytosolic aggregates in motoneurons. Interestingly, overexpression of both $hVAPB^{wt}$ and $hVAP\text{-}B^{P56S}$ induces death of motoneurons but has no effect on cortical and striatal neurons survival. This selective death of motoneurons correlates with an increased susceptibility of motoneurons to ER stress as elicited by thapsigargin. We show that VAP-B-induced motoneuron death requires activation of p38 kinase, the ER-associated caspase-12 and the effector caspase, caspase-3. Additionally, we observe that the VAP-B killing effect involves a Ca^{2+}-dependent pathway acting through calpain activation. We also observed that expression of $hVAP\text{-}B^{P56S}$ or $hVAPB^{wt}$ in motoneurons leads to fragmentation of the Golgi apparatus. These data suggest that overexpression of either $VAP\text{-}B^{WT}$ or $VAP\text{-}B^{P56S}$ leads to death of motoneurons by disturbing the ER homeostasis. We are currently investigating molecular mechanisms of motoneuron susceptibility to ER stress elicited by VAP-B.

P52 EFFECT OF MITOCHONDRIAL SUBSTRATES ON CELL SURVIVAL AND ROS GENERATION IN AN *IN VITRO* MODEL OF PROPOSED MECHANISMS OF ALS PATHOGENESIS

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Keywords: neurotoxicity, neurorescue, cell death

Background: Metabolic changes and mitochondrial dysfunction have been implicated in the pathogenesis of ALS and other motor neuron diseases. We have shown that there are intrinsic differences between brain (BM) and spinal cord mitochondria (SCM) in relation to respiratory substrates, which become even greater in the presence of mutant SOD1. These observations suggest that metabolic differences between BM and SCM may be both of pathogenic and therapeutic significance for ALS.

Objective: The ability of mitochondrial substrates to rescue a motor neuron-like cell line from cell death in our cell-based model of ALS pathogenesis utilizes neurotoxic agents with mechanisms of action similar to the pathogenic mechanisms seen in ALS. Based on our *in vivo* work with SOD1 rats, we studied the effects of glutamate, pyruvate and malate, alone or in combination, on cell viability and reactive oxygen species (ROS) generation in this sporadic ALS model system.

Methods: The toxicity of glutamate, pyruvate or malate was examined in differentiated NSC-34 cells. Cell death was assessed after a 24 hour (hr) exposure to doses ranging from 0 mM to 7.5 mM for each substrate. We examined whether glutamate, pyruvate and malate could improve cell survival of NSC-34 cells following (1) staurosporine-induced caspase-3 activation, (2) thapsigargin-induced endoplasmic reticulum Ca^{2+} ATPase inhibition, (3) carbonyl cyanide chlorophenyl hydrazone (CCCP)-induced mitochondrial uncoupling and (4) hydrogen peroxide-induced free radical production. NSC-34 cells were exposed to the mitochondrial substrates (singly, in pairs or as a triple combination) simultaneously with each of the neurotoxic agents listed and then assayed at 24hr for viability. Cell death was determined using nuclear staining and morphology with Hoechst 33342 and propidium iodide.

Results: Mitochondrial substrates were not toxic to NSC-34 cells using a nuclear morphology staining assay. NSC-34 cells were then simultaneously exposed to the substrates (alone or in combination) and either thapsigargin or hydrogen peroxide for 24hrs. The triple combination of glutamate, pyruvate and malate improved viability when given simultaneously with hydrogen peroxide as compared to the inducer alone (increased by 58.8%; $p \leq 0.05$). Only the combinations containing pyruvate or pyruvate alone showed improved survival (increased by 59–63%). No significant improvement in cell survival was observed with thapsigargin, although there was a trend towards improvement with addition of the triple combination (increased by 11.6–13.6%). Additional studies examining the other neurotoxic agents and effects on ROS generation are currently underway.

Conclusions: Mitochondrial substrates, glutamate, pyruvate and malate, particularly pyruvate, improve differentiated NSC-34 cell survival following hydrogen peroxide-induced cytotoxicty compared with the effects of these substrates on other toxicological mechanisms and warrant further study as potential therapeutic agents.

P53 ACTIVATION OF THE HEAT SHOCK RESPONSE IN A CELLULAR MODEL OF ALS

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Keywords: Heat shock response, arimoclomol, celastrol

Background: Heat shock proteins (Hsp) are chaperones that aid protein folding during protein synthesis and under conditions of stress Hsps protect cells from stress. Although up-regulation of Hsps rescues motoneurons in a mouse model of ALS, the relationship between increased Hsp expression and neuronal survival is not straightforward (1,2). In SOD1 mice, over-expression of Hsp70 has no beneficial effects (3) whereas overexpression of Hsp27 offers marginal benefits (4). However, induction of multiple Hsps is more effective than individual family members (5,6). Some inducers of Hsps, such as arimoclomol and celastrol act on the transcription factor of Hsp, HSF-1, thereby achieving induction of members the whole heat shock response (HSR) machinery (7,8)

Objectives: We compared the neuroprotective and hsp inducing effects of two inducers of hsp expression, celastrol and arimoclomol following exposure of primary motoneurons to apoptotic stimuli *in-vitro.*

Methods: Primary rat motoneuron cultures were obtained (9). Cells were exposed to either 200 nMS taurosporin or 100 μM H_2O_2 and upregulation of Hsps was achieved by co-treating some cultures with either arimoclomol (0.1–100 μM) or celastrol (0.01–3 μM). The effects on motoneurons were assessed by immunostaining for the inducible Hsp70 and activated caspase-3. Motoneuron survival was assessed by counting surviving MAP-2 cells.

Results: H_2O_2 or Staurosporin treatment leads to the activation of apoptosis, and treated cells show increased

expression of activated caspase-3. Treatment of stressed cells with hsp inducers induces expression of Hsp70. However, although both arimoclomol and celasterol induce Hsp70, their neuroprotective effects are very different. Thus, whereas arimoclomol protects motoneurons from apoptotic death at a concentration of 1 μM, celastrol showed no neuroprotective effects at any dose applied.

Discussion: Results indicate that not all agents that activate the HSR will necessarily have neuroprotective effects. Furthermore, the mechanism of action of arimoclomol may involve activation of other coupled mechanisms that interfere with the apoptotic pathway, a mechanism that is not activated in celastrol treated cells.

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P54 INTERACTIONS BETWEEN SOD1 AND SMALL HEAT SHOCK PROTEIN 22

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Keywords: heat shock protein, SOD1, chaperone

Background: Amyotrophic lateral sclerosis (ALS) is a relentlessly progressive neuromuscular disease characterized by progressive loss of motor neurons in the brain and spinal cord. A proportion of familial cases are due to mutations in the gene for SOD1. Whilst the mechanisms underlying mutant SOD1-mediated cytotoxicity are currently unknown, a possible mechanism may be an impairment in the protein quality control machinery of motor neurons. Heat shock protein 22 (Hsp22/HSPB8) is a molecular chaperone belonging to the small heat shock protein family which is selectively expressed in motor neurons and mutations affecting residue 141 of the Hsp22 protein lead to hereditary motor neuropathy.

Objectives: To determine whether Hsp22 levels are altered by mutant SOD1 and whether any interaction may be relevant to SOD1 mediated neuronal cell death

Methods: NSC-34 cells stably overexpressing SOD1 mutants G37R, and G93A, were created and Hsp22 transcript and protein levels were measured by rtPCR and Western blotting respectively. The effect of transfection of wild type and mutant Hsp22 on SOD1 aggregate formation was assessed by fluorescence microscopy.

Results: Western blots showed a decrease in Hsp22 protein expression compared to cells expressing wild type SOD1. In contrast to Hsp22 protein levels, Hsp22 mRNA, as determined by RT-PCR, remains unchanged suggesting that the decrease in Hsp22 protein levels occurs post-transcriptionally. The use of proteasome inhibitor, MG132, was found to restore Hsp22 levels in mutant SOD1 expressing NSC-34 cells to levels comparable to wild type (WT) SOD1 expressing cells, suggesting that mutant SOD1 may specifically modulate proteasome activity. Over expression of wild type Hsp22 suppressed the formation of SOD1 protein aggregates while expression of K141N mutant Hsp22 led to enhanced aggregate formation.

Conclusions: Hsp22 displays a pattern of expression which is consistent with a potential role as a motor neuron specific chaperone. Expression studies in neuronal cells stably expressing mutant and wild type SOD1 indicate that mutant SOD1 may exert a specific effect in down regulating Hsp22 levels via the proteasome. Conversely, mutant Hsp22 may be deficient in chaperoning SOD1 and preventing the formation of insoluble protein aggregates.

P55 MANIPULATION OF THE HEAT SHOCK RESPONSE IN MSOD1 ASTROCYTES

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Background: Although the precise mechanism by which the mutant SOD1 (mSOD1) results in selective motoneuron degeneration remains unclear, it has recently become clear that mSOD1 toxicity is not restricted to expression within motoneurons alone. Several lines of evidence have shown that the expression of mSOD1 in non-neuronal cells such as astrocytes, microglia and oligodendrocytes plays a critical role in motoneuron degeneration in mSOD1-ALS (1–5). In view of the role that non-neuronal cells play in ALS pathogenesis, it is possible that targeting these cells may be a successful approach in the development of a therapeutic strategy for ALS. In particular, exploitation of endogenous defense mechanisms that exists within these different cell types, particularly in astrocytes, may be effective. The heat shock response (HSR) is one such ubiquitious cellular defense mechanism that involves the expression of a family of proteins called heat shock proteins (Hsps). These proteins are molecular chaperones that carry out a range of house-keeping functions and protect cells from a variety of cell stress. Therefore, a better understanding of the HSR in different cell populations may help to establish whether this mechanism may a targeted as a therapeutic approach in ALS.

Objectives: To establish the effect of expression of mSOD1 in astroglia on various components of the HSR in astroglia from different regions of the CNS and to evaluate the effect of upregulation of the HSR in astrocytes on motoneuron survival.

Methods: In established *in vitro* cultures of isolated primary astrocytes cultures from cortex and spinal cord of $SOD1^{G93A}$ transgenic mice, elements of the HSR will be analysed. These assessments will involve immunocytochemistry, and Western blot analysis. Hsp expression in WT and mSOD1 astrocytes will be upregulated by treatment with agents that are known to upregulate Hsp expression, and the effect on the survival of co-cultured primary WT and mSOD1 motoneurons will be assessed.

Results, Discussion and Conclusions: We found that astrocytes from SOD1-G93A mice have regional specificity in which cortical mSOD1 astrocytes are less activated than spinal astrocytes, as shown by reduced level of GFAP expression, NO level production and iNOS expression. This indicates that a regional difference in mSOD1 toxicity in astrocytes exists. This heterogeneity among astrocytes suggests that their role in motoneuron survival is more complex than is commonly recognized. In addition, under basal

conditions, mSOD1 expressing astrocytes have a much reduced stress response compared to WT astrocytes indicated by reduced levels of expression of a number of Hsps. This reduced stress response in spinal astrocytes of SOD1 mice may play a role in ALS pathogenesis.

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P56 NEURON-GLIA INTERACTIONS UNDERLYING AXONAL HEALTH IN AN *IN VITRO* SPINAL CULTURE MODEL OF RELEVANCE TO ALS

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Keywords: spheroid, glia, neurofilament

There is increasing evidence that axonal degeneration underlies initial motor dysfunction prior to motor neuron loss in ALS, and that axonal protection may be an important target for future therapeutic intervention. Several studies have demonstrated the importance of non-neuronal cells in the pathogenesis of ALS and the progression of the disease is accompanied by changes in the glial cell populations including astrogliosis and alterations in progenitor cells. However, there is little information on how these changes affect the health of the axon. Using co-cultures of primary spinal motor neurons and mixed glial cell populations we have investigated how the environment provided by non-neuronal cells affects axonal health. Spinal motor neurons were derived from E13 mice embryos, purified on an optiprep™ density gradient and yielding a subset of motor neurons immunopositive for SMI32, an antibody to de-phosphorylated neurofilaments. Motor neurons were plated onto confluent mixed glial feeder layers derived from P3 mice. In some instances feeder layer cells were derived from G93A mSOD1 mice or were pre-aged for 9 weeks prior to plating of motor neurons. Spinal motor neurons were also plated onto purified astrocytes and meningeal cells. Co-cultures were maintained for up to 21 days, followed by paraformaldehyde-fixation and immunolabelling for cytoskeletal proteins, glial markers, cytochrome c and ubiquitin.

Mixed glial feeder layers consisted predominantly of astrocytes, fibroblasts, microglia, and progenitor cells (GFAP, fibronectin, ferritin and nestin immunoreactivity, respectively). Spinal motor neurons grown on mixed glial feeder layers developed swellings in the proximal portion of the axon, which were up to 40 μ m in diameter. These axonal swellings *in vitro* were morphologically similar to axonal spheroids in spinal cord tissue from 20 week G93A mSOD1 mice. Swellings in both mSOD1 mice and cultured motor neurons were immunolabelled for neuronal intermediate filament proteins and a subset in both models were immunoreactive for cytochrome c and ubiquitin. The size of the axonal swellings *in vitro* was increased with the age of the neuron. The density of swellings was influenced by the phenotype of glial feeder layer, with a significant ($p < 0.05$) increase being induced by a glial feeder layer that was pre-aged prior to plating the motor neurons, or expressing mSOD1. However, 84% swellings were present in areas of the coverslip that were devoid of GFAP –positive astrocytes. These data indicate that the formation of proximal axonal swellings in cultured spinal motor neurons is influenced by non-neuronal cells, and the phenotype and health of these cells affects the severity of this pathology. This model of proximal neurofilament-rich swellings in cultured neurons supports a role for disrupted neuron-glia interaction in the pathogenesis of ALS.

P57 MUSCLE FIBRES EXPRESSING MSOD1 DISPLAY ELEVATED NOS ACTIVITY *IN VIVO* AND *IN VITRO*

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Keywords: Nitric oxide synthase, fast-twitch, skeletal muscle

Background: Non-neuronal cells in the CNS are known to play a critical role in ALS pathogenesis. It is possible that cells such as muscle, which interact with motoneurons in the periphery, may also influence disease progression. Muscle atrophy is a characteristic feature of ALS, and is associated with increased mitochondrial reactive oxygen species (ROS) and nitric oxide (NO) production, resulting from denervation (1). NO may have deleterious effects on the muscle function and can act to inhibit force production (2). In the Wobbler mouse model of motoneuron degeneration, inhibition of NO synthesis improves motor function and delays motoneuron death (3). Neuronal NOS (nNOS) is expressed at high levels in fast twitch myofibres in normal adult mice (2). However, the role of muscle nNOS in the progression of ALS is not clear.

Objectives: To investigate whether the expression of mutant SOD1 induces changes in NO production in primary myotubes cultured from neonatal wild type (WT) and SOD1 hindlimb muscles, under basal conditions as well as in a model of exercise-induced stress. We also compared nNOS expression in fast and slow muscles of WT and symptomatic SOD1-G93A mice and examined the relationship between nNOS expression and NO production in slow and fast muscle cells cultured from adult mice.

Methods: Satellite cells of neonatal and adult hindlimb muscles were cultured. Exercise related stress was modelled by glucose deprivation combined with caffeine (1–20 mM). NO levels were measured from cell supernatants using the modified Greiss Reagent assay.

Expression of nNOS was examined in Soleus and EDL muscles of WT and endstage SOD1-G93A mice by immunohistochemistry.

Results: Following exposure to exercise-related stress, in WT muscle cultures there was a dose-dependant increase in NO production in response to glucose deprivation and caffeine treatment, with a 3 fold increase in NO levels after 24 hours. However, in satellite cells of neonatal SOD1 mice, NO production was elevated, even in the absence of stress and these cells responded to stress at a faster rate and to a greater extent than WT cells. In muscle sections from WT and SOD-G93A mice, there was a disease-specific increase in the expression of nNOS in fast muscles and to a lesser extent in

slow muscles. Similarly, in culture, adult fast muscles of SOD1-G93A mice released more NO than either WT muscles or slow muscles of SOD1-G93A mice.

Discussion: *In vitro*, SOD1 muscle cells intrinsically produce more NO than WT muscle cells. SOD1 muscle cells are also particularly sensitive to exercise-related stress and have an enhanced NO response, which is disease-stage and muscle-type dependant. These characteristics of fast muscles of SOD1 mice, may contribute to their differential vulnerability to disease in ALS.

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P58 DIFFERENTIATION OF A FAMILIAL ALS CELLULAR MODEL ALLOWS DETECTION OF ALTERED PROTEIN EXPRESSION IN THE SOD1 TRANSFECTED CELL LINES

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Keywords: SH-SY5Y, differentiation, cdk5/p35

Background: The cdk5/p35 pathway has been suggested to be involved in ALS pathogenesis. Deregulation of the Ser/Thr kinase cdk5 activity has been demonstrated in a transgenic mice model of ALS (1) as its involvement would be associated with hyperphosphorylation of NF-H and tau observed in ALS patients and mice. Deregulation of cdk5 correlates with the increased generation of a truncation product of the cdk5 regulatory unit p35, termed p25. Preliminary experiments performed using an undifferentiated SOD1-mutated transfected neuroblastoma cell line proved that there were no modifications in the cdk5/p35 pathway and in phosphorylated tau. In our laboratory, the treatment of the SH-SY5Y human neuroblastoma cell line with the differentiating agents, retinoic acid and brain derived neurotrophic factor (BDNF), provided morphological and expression evidences of neuron-like phenotype.

Objectives: To evaluate the presence of modified expression of the cdk5/p35 proteins and its substrate, tau, after inducing differentiation of the SOD1-mutated transfected neuroblastoma cell line.

Methods: The human neuroblastoma SH-SY5Y cell line was transfected with the wild-type (WT-SOD1) and the G93A-mutated (G93A-SOD1) SOD1 gene. The un-transfected and transfected cell lines were cultured and treated with *All-trans*-retinoic acid at a final concentration of 10 μM in DMEM with 15% foetal bovine serum for 5 days and afterwards with 3.75 μM BDNF in serum free DMEM for 7 days. Western blot was performed with anti—actin, -p35, -cdk5, -tau, -tau phosphorylated at ser 202 and thr 205. The expression of neuron-specific markers, tau and tubulin beta 3, was assayed by florescence microscopy, in differentiated compared with undifferentiated cells.

Results: The treatment with the differentiation agents induced a morphological change consisting of a more neuron-like phenotype. Almost all treated cells had long bipolar or multipolar processes and neurite extension that occasionally connected the cells. The results showed an increased expression of tau and tubilin beta 3. The western blotting experiments demonstrated that, respectively from undifferentiated cell lines, the WT- and G93A-SOD1 mutated cell lines had a significantly higher p25/p35 protein ratio compared to untransfected cell line ($p < 0.05$). Moreover, the G93A SOD1-mutated cell line was significantly hyperphosphorylated in the ser 202 site of tau compared to base cell line ($p < 0.05$).

Discussion: Our results prove that the undifferentiated neuroblastoma cell line is not a suitable model to study the mechanisms of ALS pathogenesis. The modified pattern of protein expression in the SOD1-transfected cell lines observed after differentiation treatment demonstrates that this model, nearer to the neuronal phenotype, may be useful to studying ALS pathogenic mechanisms.

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THEME 4 EPIDEMIOLOGY & GENETICS

P59 ANALYSIS OF PHYSICAL ACTIVITY AS A RISK FACTOR FOR MOTOR NEURONE DISEASE

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Keywords: Physical activity, risk factor, questionnaire validation

Background: Reports suggesting a higher incidence of motor neurone disease (MND) in professional sports people have lead to speculation of an aetiological relationship between physical activity (PA) and the development of MND (1, 2) This is supported by hypotheses regarding the underlying cellular mechanisms of MND, in particular oxidative stress and glutamate excitotoxicity. However, definitive evidence is lacking, with prior studies plagued by methodological problems and conflicting results.

Aims and Objectives: We hypothesise that a high level of previous PA is associated with an increased risk of MND in individuals with an underlying genetic susceptibility. Our objective is to create and validate a new adulthood PA questionnaire which can be used to evaluate PA as a risk factor for MND.

Methods: In collaboration with a unit who have expertise in PA epidemiology, we have designed a novel questionnaire to collect data regarding PA performed from aged 20 years onwards. Using closed questions, we ask

about PA performed at home, work and leisure, structured into time periods of 5 or 10 years. To validate the questionnaire we conducted 100 interviews using participants from a large population-based cohort created by the epidemiology unit to prospectively investigate factors linked to diabetes. Self-reported durations of PA were quantified into energy expenditure scores using an established coding scheme which classifies activities according energy expenditure.(3) These were compared with objective PA energy expenditure measurements taken from cohort members 10 and 15 years ago, calculated using the 4-day heart-rate monitoring technique with individual calibration.(4) The degree of correlation between objective and subjective measurements of PA was determined using the Spearman's rank correlation coefficient.

Results: A high degree of correlation between the questionnaire and objective PA variables was achieved. Correlation between total self-reported PA scores and objective PA energy expenditure was 0.34 for PA 10 years ago ($p < 0.001$) and 0.47 for PA 15 years ago ($p < 0.001$).

Discussion, Conclusions and Future Work: This novel adulthood PA questionnaire displays a level of validity beyond that reported in previous similar questionnaires. It will be invaluable in the analysis of PA as a risk factor for MND, providing a practical instrument for accurate quantification of historical PA and therefore addressing many of the challenges encountered in previous studies. As such, we will conduct a retrospective case-control study using incident MND cases and matched community controls without neurological diagnoses. Differences in PA participation between cases and controls will be analysed and any association between MND and PA determined.

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P60 TOXICITY OF β-N-METHYLAMINO-L-ALANINE TO AQUATIC ORGANISMS

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Keywords: Neurotoxicity, Animal models, BMAA

Background: The toxicity of β-N-methylamino-L-alanine (BMAA) a novel cyanobacterial amino acid, has been established over the past 40 years in a range of vertebrate models. The mechanism of BMAA toxicity has been determined *in vitro* in dissociated mixed spinal cell cultures, showing selective motor neurone loss (1) and with other insults, a potentiation of neuronal injury at 10μM BMAA (2).

The widespread production of BMAA by symbiotic and free-living cyanobacteria (3) raises questions of BMAA toxicity to aquatic organisms. Aquatic models are needed due to the risks of exposure of aquatic biota to BMAA, and their roles as both indicators of effects on community health and as primary cyanobacterial consumers in the food chain.

The Brine Shrimp, *Artemia salina* and Zebra Fish *Danio rerio* are valuable models for toxicity assessments and have, for example been used to establish toxicity of cyanotoxins, as well as to assess toxic effects on communities at large. In addition, much work has been done outlining neuronal developmental pathways and *D. rerio* is proving to be a valuable model in neuro-degeneration research (4).

Early stages of neuronal development are highly conserved among vertebrates; *D. rerio* is an ideal model organism to better understand the mechanisms associated with BMAA-induced motor neurone degeneration, as the homologous nature of early stage neuronal development across species, means that *D. rerio* analysis identifies common developmental principles, particularly neurone patterning, signalling pathways and the locomotor regions

Objective: To determine the toxicity of BMAA to aquatic organisms *A. salina* and *D. rerio*.

Methods: Multiple methodologies were used to assess the effects of BMAA exposure, including a 72-hour survival bioassay and 5-day behavioural assay for *A. salina*, and a 6-day bioassay for *D. rerio*, monitoring developmental, morphological-, and behavioural endpoints.

Results: BMAA caused time-and concentration-dependent mortality and loss of photo-tactic response in *A. salina*. Early stages of *D. rerio* development were more susceptible to

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BMAA, embryo hatching was delayed, and larval mortalities occurred due to BMAA exposure. BMAA reduced larval heart rate and induced oedema over a 6-day developmental period, during which neuro-muscular deformities occurred, including: occular deficiency, head deformation and trunk deformities.

Conclusions: The lowest observable adverse effect level (LOAEL) in both *A. salina* and *D. rerio* larvae (5μg/L; 42nM), occurred at environmentally-relevant BMAA concentrations. The increased sensitivity of *D. rerio* to BMAA indicates that it may indeed serve as a useful model to further investigate BMAA- induced neuro-degeneration; and that the bioavailability and neuro-toxicological significance of BMAA to aquatic biota require further investigation.

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P61 A CLUSTER OF AMYOTROPHIC LATERAL SCLEROSIS IN CLOSE PROXIMITY TO TWO LAKES IN ENFIELD, NH, USA WITH KNOWN BLOOMS OF POTENTIALLY TOXIC CYANOBACTERIA

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Keywords: epidemiology, cyanobacteria, environmental risk factors

Background: Cynaobacteria are associated with many neurotoxins and cytotoxins including β-methylamino L-alanine (BMAA), anatoxin (ATX), saxitoxin(STX), anatoxin a(S), curacin, microcystins and cylindrospermopsin (CYL). All of the neurotoxins have targets on the central or peripheral nervous system. BMAA has been linked to neurodegenerative disease as with amyotrophic lateral sclerosis/parkinsonism–dementia complex (ALS/PDC) that has been remarkably prevalent amongst the Chamorro people of Guam.

Methods: Meticulous review of patient records at our institution was undertaken to substantiate the diagnosis of sporadic ALS and document the exact dwelling address, family history and other relevant social and medical histories. Methods for examining water samples included phycological, chlorophyll and turbidity analysis. Liquid chromatography-mass spectrometry (LCMS) was used to measure free BMAA, ATX, STX, microcystins and CYL from filtered water samples.

Results: Water samples collected from Lake Mascoma in November 2007 were confirmed by microscopic examination to contain high numbers of potential-toxin producing cyanobacteria. However, we were unable to detect significant levels of any BMAA, microcystin, CYL or ATX in filtered samples of lake water using LCMS. Epidemiological evaluation of the geographical area of Lake Mascoma and a small adjoining Crystal Lake in Enfield, NH (population 4,854 in 2006; 120.5 persons per square mile of land area.), together encompassing an area of approximately 7.75 square miles (2.75 square miles of inland water excluded) shows an incidence of sporadic ALS that is approximately 25 times the expected incidence of 2/100,000/year. The 8 ALS patients who lived on or near the lakes (on average less than 0.15 miles from the shore) for a minimum of 9 years, were diagnosed between the years 1991–2007. Documented blooms of cyanobacteria have been reported in Lake Mascoma previously as part of a state monitoring program.

Discussion: We postulate that the high incidence of ALS in this patient cluster could be directly related to the chronic exposure of the cyanobacteria producing neurotoxins. Our inability to detect toxin levels in our samples may be a technical issue (we only analyzed from free BMAA and free microcystins, not the bound components) or may reflect the episodic occurrence of toxic algal blooms. We conjecture that the exposure might be related to aerosolization of toxin as with some other toxic algae; however the exposure could be potentially through other means such as swimming, eating fish from the lake or direct ingestion of water.

P62 THE NEUROTOXICITY OF BETA-N-METHYLAMINO-L-ALANINE (BMAA) IN *DROSOPHILA*

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The neurotoxic amino acid BMAA is produced by known groups of cyanobacteria in terrestrial, freshwater and marine environments, suggesting a potential for wide-spread human exposure. Evidence of such exposure has been demonstrated in brain tissues from patients from Guam with Amyotrophic Lateral Sclerosis (ALS)/Parkinsonism dementia complex and North American patients with ALS and Alzheimers disease (1). Neuronal culture (*in vitro*) and primates/rodent model (*in vivo*) experiments have provided some insights to the neurodegenerative properties of BMAA.We used the fruit fly *Drosophila melanogaster* as a model to investigate the effects of L-BMAA *in vivo*. We first conducted survival assays (1–2 day-old wild type flies were fed with a medium containing 0/2/4/6/8/10 mM BMAA). Another two amino acids, glutamate and alanine, were also used in similar concentrations to evaluate the toxicity of BMAA. We found that BMAA dramatically reduced the life span of flies in a dose dependent manner. Interestingly, glutamate also reduces lifespan but the effect is much less severe compared with BMAA at the same concentration. Alanine has no effects on the life span of flies. To compare the toxicity of BMAA with other environmental toxins, we used an herbicide paraquat in our assay. Paraquat is a reactive oxygen species inducing compound that causes neurodegeneration. We found that the toxicity of 4 mM BMAA is comparable with that of 2 mM paraquat. Second, we used negative geotaxis assays to evaluate the motoneural coordination and the integrity of the nervous system. The geotactic behavior was significantly affected in flies exposed to low (2mM) concentrations of BMAA and the effects are more severely affected in higher concentrations. Third, we evaluated the neuropathological changes using immunofluorescent confocal microscopy. Interestingly, we observed a possible up-regulation of pHistone in the brain of adult flies fed with

BMAA. We demonstrated the toxic effects of BMAA on life span, motor function and neuronal function/morphology. The dramatic effect of BMAA on the nervous system that we observed in *Drosophila* not only provides strong evidence for an environmental cause for neurodegenerative disease but also validates and exemplifies the power of *Drosophila* as a model system.

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P63 CYANOBACTERIA AND BMAA: POSSIBLE LINKAGES WITH AVIAN VACUOLAR MYELINOPATHY (AVM) IN THE SE UNITED STATES?

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Keywords: Cyanobacteria, BMAA, AVM

Background: Avian vacuolar myelinopathy (AVM) is a neurological disease that produces uncoordinated behavior in affected waterfowl. AVM is characterized by bilaterally symmetrical vacuolation of the white matter of the brains and spinal cords of AVM-affected birds. Since 1994, AVM has caused the deaths of >100 bald eagles (*Haliaeetus leucocephalus*) and thousands of American coots (*Fulica americana*) at numerous sites in the southeastern (SE) United States. AVM is now believed to be the most significant unknown cause of eagle mortality in the history of the United States. Despite extensive screening for a wide rage of pathogens and toxins (prions, organic metals, pharmaceuticals and plant toxins), the causative agent for AVM has not been identified. More recently, feeding and sentinel trials, field surveys, and genetic studies have implicated exotic *Hydrilla verticillata* and an associated epiphytic cyanobacterial species (Order Stigonematales) as a causal link to AVM. This undescribed Stigonematales species covers up to 95% of the surface area of *Hydrilla* leaves in reservoirs where AVM-related bird deaths have occurred.

In order to explore possible linkages between BMAA and AVM, a variety of tissue samples were screened for BMAA. Samples included cultures of Stigonematales grown on BG-11 medium (with and without nitrate), *H. verticillata* minus the Stigonematales epiphyte, and *H. verticillata* plus the Stigonematales epiphyte. Samples were hydrolyzed and analyzed by HPLC/FL. BMAA was detected in the Stigonematales (plus NO_3^-) culture, the Stigonematales (minus NO_3^-) culture (30 μ g g^{-1} dry weight), and the *Hydrilla*/Stigonematales field-collected sample (59 μ g g^{-1} dry weight); BMAA was below the limit of detection (5 pmoles per injection) in the *Hydrilla* field-collected sample. The identification of BMAA in the *H. verticillata* plus the Stigonematales epiphyte sample was confirmed by LC/MS. We hypothesize that the BMAA concentration in the field-collected Stigonematales was greater than 59 μ g g^{-1} since it "diluted" with non-BMAA containing *H. verticillata* biomass.

If BMAA is biomagnified in these wetland ecosystems, as has been observed in Guam, then the consumption of fish (e.g., shad and herring) and waterfowl (e.g. Canada geese and mallards) from local reservoirs could represent a significant health risk for humans. This risk also applies to other regions in the SE United States as AVM-positive sites have been identified in Arkansas, Texas, Georgia and North Carolina. Future studies will assess the degree of biomagnification of the neurotoxic amino acid, BMAA, in wetland ecosystems of the SE United States.

P64 CYANOBACTERIAL TOXIN BMAA IN NEURODEGENERATION

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Keywords: BMAA, environmental toxin, pathogenesis

Background: Cyanobacteria are found in almost every conceivable habitat, from oceans to fresh water to bare rock to soil. Most are found in fresh water, while others are marine, occur in damp soil, or even temporarily moistened rocks in deserts. The neurotoxic amino acid beta-N-methylamino-L-alanine (BMAA) is produced by laboratory strains isolated from all five known morphological groups of cyanobacteria, including cyanobacterial symbionts and diverse taxa of free-living cyanobacteria. The ubiquity of cyanobacteria in terrestrial, as well as freshwater, brackish, and marine environments, suggests a potential for wide-spread human exposure. BMAA is a naturally occurring, non-protein, non-essential amino acid that is found in high concentrations in brain tissues of patients with Amyotrophic Lateral Sclerosis-Parkinsonism-Dementia Complex (ALS/PDC) in the South Pacific island of Guam. More recently, BMAA has been measured in cohorts of Caucasian, North American patients with sporadic Alzheimer's disease and ALS. Although the role of BMAA in human degenerative disease is highly debated, there is evidence to suggest that BMAA may mimic glutamate toxicity.

Objective: To demonstrate human exposures to the neurotoxic amino acid beta-*N*-methylamino-L-alanine (BMAA) are associated with neurodegenerative disease. The neurotransmitter glutamate is an excitotoxin that has been implicated in a number of different neurodegenerative diseases. We report here that BMAA is detected in an independent cohort of ALS and AD cases that came to brain autopsy.

Methods: BMAA was measured in cryopreserved brain specimens taken from the cerebral cortex of ALS (N = 12), AD (N = 13) and age-matched control subjects (N = 12). BMAA was quantified in brain in protein-bound fractions using a validated fluorescence HPLC method confirmed by tandem quadrupole and Quadrupole time of flight (Q-ToF) mass spectrometry. We conducted biodistribution and *ex vivo* autoradiographic studies of BMAA in rodents, using custom synthesized (^{3}H)BMAA.

Results: In human postmortem brain, BMAA was measured in varying concentrations as a bound component in archived brain tissues sampled from the frontal and temporal cortices. BMAA concentrations ranged from 30 to 250 μg/g in ALS (134 μ g/g +/− 12.8 (mean +/− SEM)) and 10 to 230 μg/g in AD patients(111 μ g/g +/− 14.6). In contrast, BMAA was only detected in one age-matched healthy control subject. In rodent studies, we observed specific uptake of radiolabeled BMAA that was localized to specific cortical and brainstem regions. Time course studies demonstrated that BMAA reached a plateau at 8 hours and remained elevated in brain at 48 hours post injection. In contrast to the brain, BMAA levels declined rapidly over time from other organs.

Discussion and Conclusions: This observation suggests that BMAA is transported into the brain and that the amino acid may be incorporated into certain target areas due to selective incorporation in proteins or by glutamate uptake mechanisms. This is the second confirmation of BMAA exposures in sporadic ALS and AD patients from North

America. These observations support a role for BMAA as a possible gene/environment interaction, serving as a potential trigger for certain types of neurodegenerative processes that share a common molecular pathogenesis but involve different anatomical regions of the brain.

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P65 MULTIPLE NEUROTOXIC DIETARY ITEMS IN THE CHAMORRO DIET: IS A LINK TO ALS/PDC PLAUSIBLE?

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Keywords: Diet, Neurotoxicity, BMAA

Background: Initial reports (1) that cycad flour in the Chamorro diet may be linked to ALS/PDC resulted in five international cycad conferences and significant debate within the literature. The claim that washed cycad flour only has low amounts of BMAA (2) has been updated by the finding of 50–100 times the amount of protein-bound compared with free BMAA in cycad flour. Recently flying foxes were added as a possible source of BMAA in the Chamorro diet (3).

Objectives: We sought to determine if there are other possible inputs of BMAA in the traditional Chamorro diet.

Methods: Using AQC precolumn derivatization and HPLC-FD with LC/MS and triple quadrupole LC/MS/MS we tested for BMAA in a variety of dietary items prepared by the Chamorro people including washed cycad flour, cycad dough, cycad tortillas, cycad dumplings, cooked flying fox, flying fox stew, and flying fox broth, as well as 21 different flying fox specimens and a sample of feral deer.

Results: Although we did not detect significant concentrations of free-BMAA in traditional foods prepared by Chamorro villagers from washed cycad flour, washing does not remove protein-associated BMAA. We did detect significant quantities of protein-bound BMAA in cycad dough (4.7 μ g/g), cycad tortillas (334 μ g/g), and cycad dumplings (84 μ g/g). Feral deer had a low amount of BMAA (3 μ g/g in hair). Flying fox broth was particularly rich in protein-bound BMAA with a 1 litre portion resulting in ingestion of 12.4 mg of BMAA. BMAA may also occur in dietary items from other ALS/PDC foci.

Conclusions: Multiple inputs of BMAA in the Chamorro diet confound epidemiological analyses based on a single dietary item (4). Thus determining whether consumption of cycad flour alone, or flying foxes alone, or even feral pigs, deer, and land crabs (which all may occasionally feed on cycad seeds) alone ignores total input of BMAA into the Chamorro diet from multiple sources. Furthermore, since BMAA is produced by symbiotic cyanobacteria in cycad roots, as well as in free-living marine cyanobacteria (5), inputs into the Chamorro diet from marine sources may also occur. (We are currently investigating BMAA in marine fish ingested by villagers in the coastal Kii ALS/PDC focus.) Recent laboratory findings of BMAA in double-blinded brain tissues of North American AD patients, with independent replication of the results by another laboratory indicate that further analysis of BMAA and its putative link to ALS/PDC among the Chamorro people is warranted.

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P66 FRUIT AND VEGETABLE INTAKE AND RISK OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: amyotrophic lateral sclerosis, epidemiology, antioxidants

Objective: Few human studies have reported the relationship between a food rich in antioxidants and the risk of amyotrophic lateral sclerosis (ALS). We therefore analysed the relationship between fruits and vegetable intake and the risk of ALS using a case-control study in Japan.

Methods: The study comprised of 153 ALS patients diagnosed by El Escorial World Federation of Neurology criteria, and 306 population-based randomly selected controls matched to the cases by age (+/−5 years), gender, and county of residence. A self-administered food frequency questionnaire was used to estimate preillness intakes of food groups and nutrients. The strength of association between ALS and a potential risk factor was assessed by calculating odds ratios (ORs) and 95% confidence intervals (CIs).

Results: A high intake of fruit and vegetables (OR = 0.61; CI = 0.39–0.95; P = 0.04 for trend) and fruit (OR = 0.57; CI = 0.33–0.98; P = 0.04 for trend)was significantly associated with a reduced risk of ALS risk. In multivariate model, all vegetables, green and yellow vegetables and other vegetable intakes were observed an inverse association, although the association was not statistically significant.

Conclusion: Our findings suggest that dietary intake of fruit and vegetables intake may play a protective role in the development of ALS.

P67 HYPOCHOLESTEROLEMIANTS OR HYPERCHOLESTEROLEMIA IS ASSOCIATED WITH A LATER ONSET OF ALS BUT NOT WITH AN INCREASED RATE OF DECLINE

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Key words: hypocholesterolemiants, treatment, cardiovascular risk factors

Background: It has recently been suggested that hypercholesterolemia may be protective regarding the neurodegenerative process. The authors tended to suggest that physicians should not prescribe or should stop statins of fibrates to an ALS patient. However, as ALS patients frequently have vascular risk factors, such a treatment withdrawal could have deleterious effect even in a rapidly evolving disorder.

Objectives: To determine whether ALS parameters are modified in a group of consecutive ALS patients recruited in our expert ALS center.

Material and Methods: We prospectively questioned ALS patients followed in our center for antecedent hypercholesterolemia and other vascular risk factors such as myocardic infarction or chronic ischemic cardiopathy. Antecedent or current statin or fibrate treatment was noted. ALS parameters were collected from clinical files (age of onset, site of onset, current disease duration and ALSFRS at the time of questioning, presence of familial ALS). At the time of analysis (April 2008), we also collected age of death and subsequent final ALS duration.

ALS parameters were compared between the groups with or without hypocholesterolemiants. We also calculated the rate of decline (ALSFRS decline/months of evolution), in order to evaluate a potential worsening of a given ALS group (with or without treatment).

Results: There were 174 sporadic ALS (SALS) patients and 15 familial ALS cases (FALS). In all treated SALS groups, mean age of onset of ALS was significant delayed: 60 years without treatment vs 64 yrs w/statins ($p < 0.03$), 65.2yrs w/fibrates ($p < 0.03$), 64.7 w/statins or fibrates ($p < 0.003$). In all treated SALS groups, the rate of decline was lower than without treatments but this was never significant: statins (0.49 vs 0.59), fibrates (0.48 vs 0.59), statins and fibrates (0.48 vs 0.62). There was no difference between the groups, for site of onset. A large majority of the patients are alive, precluding a reliable analysis regarding prognosis, but a Cox model will be processed every 6 months until mid-2008 to evaluate this point.

In FALS, the small number of patients restricted statistical analysis. Eleven patients belonged to the untreated group with mean age of onset of 48.5yrs and a rate of decline of 0.32. In the treated group of 4 patients, mean age of onset was 61.5yrs and the rate of decline was 0.93. Both mean age of onset and rate of decline were statistically significant ($p < 0.05$ and $p < 0.003$, respectively).

Discussion: Treatment with hypocholemiants in associated both in SALS and FALS with a significant later onset. This has also been suggested recently in a large cohort of patients with Parkinson's disease. The same pattern was present in the Canadian cohort presented in the 18th International Symposium on ALS/MND. In SALS, the largest group of our cohort, there is a lower but not significant rate of decline of ALSFRS in patients with statins and/or fibrates. Our data is supportive of a protective role of either hypocholesterolemiants or hypercholesterolemia regarding ALS. However, these data cannot support a worsening effect of the treatment with either statins or fibrates in SALS.

P68 DYSLIPIDEMIA IN ALS: NO POSITIVE EFFECTS ON SURVIVAL IN A TWO LARGE ITALIAN CLINICAL SERIES

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Keywords: hyperlipidemia, survival, cholesterol

Background: Recently hyperlipidemia has been found to positively influence survival in a series of ALS patients. This observation has been related to possible effects of metabolic changes during the course of the disease.

Aims: To evaluate in a series of ALS patients the serum lipid concentrations and their influence on patients survival.

Methods: Patients from two ALS referral series in Torino and Pavia, Italy, have been considered. The patients have been seen after January 1, 2000 and prospectively followed-up. Serum lipid concentrations are evaluated as a part of the systematic blood sampling at the time of diagnosis. No patient was consuming lipid-lowering drugs at the time of the assay. Survival times have been calculated as the duration between the onset of first symptoms and death or tracheostomy or the last day of follow-up (February 29, 2008).

Results: A total of 573 patients (mean age at onset, 61.8 (SD 11.8)) with definite or probable ALS were included in the study. The mean total level of cholesterol was 2.2 ($\pm$0.4) g/L, the mean level of triglycerides was 1.2 ($\pm$0.6) g/L, the mean level of HDL was 0.6 ($\pm$0.1) g/L, the mean level of LDL was 1.3 ($\pm$0.4) g/L, the mean LDL/HDL ratio was 2.4 ($\pm$0.8). The median survival from onset was 2.9 years. No difference could be found in survival for any of the lipids variables. In particular, using a LDL/HDL ratio cut-off of 2.99 the group with higher ratio showed only a slight, not significant, increase of survival (median survival, LDL/HDL ratio <2.99, 38.5 ± 4.5 months; ≥ 2.99, 42.7 ± 5.3 months). The LDL/HDL ratio was not significant also in Cox's multivariable analysis.

Conclusions: We could not find any significant effect of hyperlipidemia in a large series of ALS patients consecutively seen in Italy. Moreover, serum lipid concentrations were generally lower in our series than in that in the cases reported and was more similar to their controls. This difference could be due to different genetic backgrounds or to different dietary habits of Italian and French populations.

Reference:

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P69 STATIN USE PRIOR TO AMYOTROPHIC LATERAL SCLEROSIS (ALS) AND PRIMARY LATERAL SCLEROSIS (PLS)

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Keywords: neuroepidemiology, statin-induced myopathy, toxicology

Objective: Define antecdent statin use in patient populations attending an ALS Clinic.

Background: Statin use, including red yeast rice, has been rarely associated with myopathy and other neuromuscular symptoms. Some patients referred to an ALS Clinic have described muscle pain and weakness following initiation of statins leading to the diagnosis of ALS.

Methods: Review of prevalence of statin use in 240 (M-153; F-87) sporadic (s) or familial (f) patients with ALS or other motor neuron disease syndromes (MNDs) seen in a Midwest university-associated ALS Clinic.

Results: Among 31/164 statin-exposed sALS patients, 11 single statin treated (SST) and 3 multiple statin treated

(MST) patients described treatment-associated pain and increased weakness before diagnosis. Only those 3 MST patients have been reported to MedWatch. In 13 SALS patient requiring thyroid (T) replacement, 1/3 SST and 2/2 MST patients had symptoms prior to ALS diagnosis. In 7 SALS patients with diabetes mellitus (DM) treated with oral agents, none required T but 1/3 SST and 1/1 MST patients had symptoms. In 4 sALS patients with DM requiring insulin, 2/4 required T but 0/2 SST and 1/2 MST patients had symptoms prior to diagnosis. Among 18 FALS patients none had DM or T and 0/2 SST patients had symptoms. Among 20 PLS patients none had DM or T and 1/2 SST and 1/1 MST patients had symptoms. There was no statin or T use, or DM among 14 dysimmune motor neuropathy/neuron disease or atypical MNDs prior to diagnosis.

Conclusions: Statin-associated pain and weakness is more common in MST SALS, hypothyroid SALS and DM SALS patients than FALS or other MNDs patients. Further studies are required to determine if statin use, particulary MST in the context of changing statins in response to statin-related symptoms is a trigger for sALS in some susceptible patients.

P70 CLINICAL AND BIOCHEMICAL INDICATORS OF VASCULAR DISEASE AND ALS RISK

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Keywords: vascular, risk, hypoperfusion

Background: A role for hypoxia and reduced vascularisation in the pathogenesis of ALS has been suggested due to observed associations of ALS and mutations in two genes involved in angiogenesis, vascular endothelial growth factor (VEGF) and angiogenin (ANG).

Objective: To assess the association between vascular risk factors and ALS.

Methods: Traditional cardiovascular risk factors (smoking, hypertension, hypercholesterolemia, diabetes, and body mass index (BMI)) and cardiovascular diseases (myocardial infarction, angina pectoris, cerebrovascular accidents, and peripheral arterial disease) prior to onset of disease were established by a questionnaire (prevalent disease, smoking, hypertension, hypercholesterolemia, diabetes and body mass index (BMI)) in 303 patients with ALS. Biochemical assessments (total cholesterol, LDL-cholesterol, HDL-cholesterol, glucose, hs-CRP, homocysteine and fibrinogen) at diagnosis were measured in blood samples of 233 patients with ALS. Comparison was made with prospectively collected data from 2100 population-based control subjects. Association with survival in ALS was also analysed.

Results: Multivariate regression analysis adjusting for age, education and smoking showed a lower body mass index (BMI) in patients (in women: OR 0.9; 95% CI 0.9–0.99; $P = 0.02$; in men: OR 0.9; 95% CI (0.8–0.9); $P < 0.001$). Age-adjusted odds ratio showed lower levels of total (in women OR 0.8; 95% CI 0.7–0.97; $P < 0.001$; in men OR 0.7; 95% CI 0.6–0.8; $P < 0.001$) and LDL- (in women: OR 0.5; 95% CI 0.4–0.7; $P < 0.001$; in men OR 0.7; 95% CI 0.6–0.8; $P < 0.001$) but higher HDL-cholesterol (in women: OR 2.6; 95% CI 1.7–4.0; $P < 0.001$; in men: OR 2.0; 95% CI 1.2–3.3; $P < 0.001$) in patients. Fibrinogen levels, measured in women only, were higher in patients with ALS (OR 5.2; 95% 3.9–6.8; $p < 0.001$). Within the patient group, no association was found between survival and biochemical indicators of vascular risk.

Conclusions: Vascular risk factors, measured clinically and biochemically, did not increase ALS risk. Lower weight and atherogenic cholesterol levels in ALS patients suggest increased ALS risk may be related to a higher metabolic rate. The implication of increased fibrinogen in female ALS patients need to be determined.

P71 ELEVATED SERUM LEVELS OF SEX HORMONES IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: Hormones, estrogen, testosterone

Background: The incidence of amyotrophic lateral sclerosis (ALS) is higher amongst men than women, with a male to female ratio varying from 1.1 to 1.8. It has been suggested that this difference points to an involvement of sex hormones in ALS. (1) This is supported by both *in vitro* and *in vivo* studies, which have demonstrated that androgens and estrogens have trophic effects on motor neurons.(2–5)

Objective: The objective of our study is to determine the relationship between sex hormone levels and ALS.

Methods: Using a case-control design, the levels of estrone (E1) and 17β-estradiol (E2) were determined in 257 men and 161 postmenopausal women with ALS and in 195 male and 105 female healthy controls. Serum levels of testosterone (T), free testosterone (FT) and sex hormone binding globulin (SHBG) were determined in 121 male and 82 postmenopausal female ALS patients and in 93 male and 55 female healthy controls. Results were adjusted for body mass index (BMI) and age using logistic regression.

Results: The mean levels of E1, adjusted for BMI and age, in both men and post-menopausal women with ALS were significantly higher than in controls (men: 195.8 nmol/l vs. 150.6 nmol/l, p value $6.04^{*}10^{-12}$, women: 211.1 nmol/l vs. 118.4 nmol/l, p value $5.38^{*}10^{-12}$). The level of T and FT in post-menopausal women with ALS was also significantly higher than in controls (T: 1.317 nmol/l vs. 0.982 nmol/l, p value 0.002, FT: 20.39 pmol/l vs. 14.63 pmol/l, p value 0.0001), but no significant difference in the levels of T and FT in male and SHBG in both sexes could be detected between patients with ALS and their controls. More results are in progress.

Discussion: Levels of E1 are elevated in male and female patients with ALS and levels of T and FT are elevated in female patients only. Elevated E1 levels amongst patients with ALS are probably the result of a secondary phenomenon, caused by adrenal dysregulation and increased peripheral conversion from adipose and muscle tissue due to muscular atrophy. Elevated levels of T and FT in female ALS patients may suggest a causal relationship between androgens and ALS, supporting the male preponderance in the incidence of ALS or it could also be the result of a secondary phenomenon. There is no clear evidence for a neuroprotective role of sex hormones in ALS.

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P72 CLINICAL AND GENETIC ANALYSIS OF A LARGE CHINESE FAMILY WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: SOD1 gene, clinical feature, prognosis

Background: Amyotrophic lateral sclerosis (ALS) is the most common and fatal degenerative motor neuron disease in adults. Approximately 20% familial ALS (FALS) have been found Cu/Zn superoxide dismutase (SOD1) gene as the causative gene, though the aetiology of the ALS is unclear.

Objective: To investigate the clinical features, progression, prognosis genetics of a big Chinese family with ALS.

Patients and Methods: All living subjects who consented for this study were examined by at least two independent neurologists for the diagnosis of ALS (according to the El Escorial and revised El Escorial criteria). They also underwent electromyographic (EMG) examination to establish lower motor neuron (LMN) dysfunction in non-symptomatic body regions. SOD1 gene mutation screening was performed by directly sequencing after informed consent.

Results: This non consanguineous Chinese family consisted of 5 generations and had an autosomal dominate inheritance pattern. Twelve patients including 9 males and 3 females were found. Ten were dead. Mean onset age was 40 year old. The initial symptom of one limb weakness was followed soon by atrophy and fasciculation in the limb and progressed to other limb, swallowing difficulty. The duration of the disease is about 1.5 years. The cause of death is breath difficulty. All the patients presented with upper motor neurone pattern such as hyperreflexia and increased muscle tone besides the lower motor neuron signs. No mutation of SOD1 gene was detected.

Conclusions: The clinical phenotype of this family is quite similar to that described in other ethnic groups. However, no mutation detected in the SOD1 gene suggests ALS is a genetic heterogeneous disease and mutation of SOD1 gene is uncommon in familial Chinese ALS.

P73 MOTOR NEURON DISORDERS IN CHINA

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Keywords: case report, Kennedy's disease, variant

Objectives: To study the clinical features of Chinese patients with motor neuron disorders.

Methods: We reviewed retrospectively 552 cases of Chinese patients with motor neuron disorders in the database of Peking University Third Hospital from 2004 to 2008. Patients with amyotrophic lateral sclerosis (ALS) whose onset age was $\geq$70 years old as well as Kennedy's disease (KD) were included. The clinical features including symptoms and sings, courses of the disease, and biochemical indicators were analyzed.

Results: A total of 26 cases of elder ALS patients (4.7%) were found. The mean age at onset was 72.3 ± 3.3 years, and gender ratio (male to female) was 2.7 *vs* 1. It was the most frequent that the first symptom was the single upper extremity weakness and atrophy (38.5%), especially the right side (26.9%), and 11.5% showed the first symptom was bulbar involvement. Among these 26 cases, 5 patients died (19.2%), 4 performed tracheastomy (15.4%), 5 manifested the clinical characteristics of "flail arm syndrome" (19.2%) which was found to have a longer median survival. The mean duration from onset to death or tracheastomy was 30.3 ± 15.3 months. In addition, it was interesting that about 34.6% of these 26 cases of ALS patients had family history of tumors, which seemed too high to explain. On the other hand, 27 KD patients were found. There was mild motor functional lesion which correlated with the course of the disease ($r = 0.77$, $P = 0.000$). The degrees of creatine kinase (920.10 ± 495.54 U/L) and triglyceride (3.27 ± 2.78 mmol/L) increased remarkably. Among them, a 27-year-old man, developed weakness of his lower limbs at age 25 and two of his three brothers have the same symptoms, was noticed that his physical examination showed all of his deep tendon reflexes were active, and Hoffman sign on the right side was positive but Babinski signs negative bilaterally. The serum level of creatine kinase is mildly increased (289 U/L). Direct sequencing showed that the exact number of CAG repeats in the patient was 52.

Conclusion: The clinical features of elder ALS patients in this study were a little dissimilar to those of typical ALS patients. Meanwhile, in KD, there were some distinctive characteristics such as elevated triglyceride level. In a young KD patient, we found his manifestation was relatively special because his age of onset was earlier and his creatine kinase level was lower than typical patients. His active tendon reflexes showed that there may have some lesions of upper motor neurons. Because of that, the patient should be differentiated with ALS carefully. We wonder whether this was a clinical variant of KD.

P74 STATUS OF CARE FOR ALS PATIENTS AND HEALTHCARE SYSTEMS IN JAPAN RESULTS OF 2006 NATIONAL SURVEY

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Keywords: the number, public support system, medical services at home

Background: In Japan, ALS patients are entitled to receive the public support system, but there are many problems to provide long-term medical care and support of patients' lives.

Objectives: ALS patients were surveyed regarding where they were cared for (home or hospital/care facility) and whether they were using a ventilator. Home-care ALS patients

who "needed sputum suctioning" and medical system providing their care were also studied to evaluate the system.

Methods: ALS patients registered at health centers in all prefectures were surveyed using a questionnaire by mail.

1: The number of ALS patients using and not using a ventilator, and numbers of patients using a ventilator who were cared for at home and a hospital/care facility were investigated. 2: Concerning home-care ALS patients "needing sputum suctioning", the gender, age, history of illness, medical care provided,and living-support services used were investigated. All data were coded and analysed using the statistical package SPSS.

Results and Discussion: The number of ALS patients registered at 400 organisations (58.4% of the 685) was 5,335, of which 1,591 (29.8%) were ventilator users. Of the 1,591, 803 (54.1%) being cared for at home.

Concerning profiles of home-cared ALS patients answers were obtained from 326 organizations (47.6% of 685), the number of home-cared ALS patients requiring "sputum suctioning" was 837, and 749(89.5% of 837, Group (A) used a ventilator, but 88 (10.5% of 837, Group (B) did not.

Gender A: 472 males (63.0%), B: 47 males (53.4%); age (mean $\pm$ SD) A: 57.96 $\pm$ 20.01, B: 63.21 $\pm$ 10.73; the duration of illness A: 3–8 years in 349 patients (46.6%), 9–18 years in 266 35.5%), B:3–8 years in 44 patients(50% of 88) and less than 3 years in 24 (27.3% of 88).

Total support for ADLwas necessary A: 706 (94.3%), B:59 (67.0%), having a specialized medical care A: 562 (75.0%) B: 76 (86.4%), having a primary care physician A: 637 (85.0%) B: 68 (77.3%), emergency visit by a physician A: 584 (78.0%), B: 53 (60.2%), emergency visit by a visiting nurse A: 636 (84.9%), B:74 (84.1%).

It is suggested that both Groups received a medical care, but not all were covered. Also, some ALS patients received care for 20 years or longer using a ventilator, so that it is necessary to sustain this care system for long time.

Conclusion: A survey of ALS patients showed that 29.8% of them were using a ventilator, and 54.1% of these ventilator users were cared for at home, and suggested problems with the securing of beds for their hospitalization, having specialized medical care, and home-visiting nursing.

P75 SPORADIC AMYOTROPHIC LATERAL SCLEROSIS IN JAPAN: AGE AT ONSET INFLUENCES ON WIDE-RANGED CLINICAL PROFILES

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Keywords: age at onset, initial symptom, Japan

Background: Symptomatic manifestations of ALS such as the frequency of various initial symptoms, their influence on clinical features, the frequency of rare symptoms such as oculomotor disturbance, the influence of age at onset on clinical features and progression, and many other symptomatic details have not been well characterised, particularly based on a large scale sample.

Objectives: To profile the detailed clinical features of sporadic ALS on large scale samples in Japan.

Methods: A nationwide registration of ALS patients has been conducted in Japan since 1974. In 2003, the registration system was revised and made available for research use. The registration of each patient is annually renewed. We analyzed 3428 newly registered cases after 2003, 4202 cases registered in a single year (2005) including those initially registered before and after 2003, and a total of 2128 cases with tracheostomy positive pressure ventilation (TPPV) from all of the registered cases. All the analysed patients were classified as definite, probable or possible ALS according to the revised El Escorial Criteria for the diagnosis of ALS.

Results: In the newly registered cases after 2003, the female to male ratio was 1.43:1 and the mean age at onset was 65.3 $\pm$ 10.7 years. In the cross-sectional view of a single year (2005), the percentage of patients using either a gastrostomy tube or a nasogastric tube was 28.7% and 7.8%, respectively. The percentage of patients with TPPV was 29.3%.

The patients with an older age at onset progressed more rapidly to the TPPV stage than those with a younger age at onset. The subpopulation of patients with long-standing TPPV showed ophthalmoplegia, while its appearance rate was less in the patients with an older age at onset than in those with a younger age at onset. Furthermore, age at onset strongly influenced the frequency of initial symptoms: dysarthria, dysphagia, neck weakness and respiratory disturbance were more frequent in patients with an older age at onset, while upper or lower limb weakness was observed more frequently in patients with a younger age at onset. In addition, the initial symptoms were still the most prominent symptoms at the follow-up stage, suggesting that the initial symptoms determine the major clinical features even in the advanced illness.

Discussion and Conclusions: Our present study demonstrated that symptomatic features are strongly influenced by the age at onset, and thus clinical features may be altered in a super-aging society.

P76 SURVIVAL AND FACTORS PREDICATING SURVIVAL IN ALS PATIENTS 15-YEARS-SURVEY PERIOD

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Keywords: ALS, survival, prognostic factor

Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder. Median survival from symptom onset is about 3.5 years, but some patients survive more than 5 years.

Objectives: To determine the mean duration of the disease and statistical probability of survival in ALS population in Belgrade during a 15-years-survey period (1992–2007).

Methods: A total of 292 (178 males and 114 females) ALS patients were discovered in the Belgrade district and were hospitalized between 1992 and 2007. All patients fulfilled El Escorial criteria for probable or definite ALS. Eighty patients had a bulbar onset and 212 had spinal onset of the disease. The outcome in 183 cases was determined by directly contacting patients or their families. 3 patients (men) could not be reached for follow-up and dropped out of the study after diagnostic hospitalization. Survival was assessed with the Kaplan-Meier method in the whole sample, by selected prognostic indicators (age, sex, bulbar or spinal onset, and disease duration). Multivariate analysis was done with the Cox proportional hazard function.

Results: As of December 2007, 220 ALS patients (75%) have died. Nineteen (7%) patients survived five years after diagnosis. The mean age at diagnosis was 59.4 ± 11.2 years. Mean survival time from diagnosis for all patients was 42 ± 4.4 months (median survival time: 24 ± 2 months). Mean age at symptoms onset was 56.8 ± 12.3 years for men, and 59.2 ± 11.1 years for women but this difference was not statistically significant. Mean age at symptoms onset was 60.3 ± 11.3 years for patients with bulbar onset, and 56.7 ± 12.0 years for patients with spinal onset of the disease and this difference was also not statistically significant. Mean survival time from symptoms onset for all patients was 70.8 ± 8.5 months (median survival time: 36 ± 2.4 months). Mean survival time from diagnosis for patients with spinal onset was 43.2 ± 4 months and 38.4 ± 5.5 months for patients with bulbar onset (Kaplan-Meier analysis, log-rank test, $p = 0.37$, no significance). Mean survival time from diagnosis for men was 46.8 ± 5 months and 37.0 ± 4.0 months for women (Kaplan-Meier analysis, log-rank test, $p = 0.43$, no significance). Mean survival time from diagnosis for patients younger than 49 years was 55.6 ± 8.4 months and 36.0 ± 3.0 months for older than 49 years (Kaplan-Meier analysis, log-rank test, $p = 0.03$, significant at 95% CI). Cox regression model was applied to adjust survival times from diagnosis for sex and age group, but hazard ratios were not found to be significant.

Conclusions: Five years' survival was 7% after diagnosis of ALS. Positive effect on survival was demonstrated in the ALS patients with the onset of the disease before the age of 49.

P76A A 10-YEAR EPIDEMIOLOGICAL PROSPECTIVE STUDY OF ALS IN PIEMONTE AND VALLE D'AOSTA, ITALY

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Keywords: Incidence, prevalence, survival

Background: Although the implementation of population-based registers has accurately measured the epidemiology of ALS in European and North American populations, it is not clear whether ALS incidence, clinical characteristics, and survival changed over time.

Aim: To assess the trend of epidemiological and clinical characteristics of ALS in two Italian regions, using a prospective population-based registry, in a 10-year period (1995–2004).

Methods: The PARALS is a prospective register collecting all cases of ALS incident in two regions of north-western Italy (total population, 4,332,842) established in 1995, based on the collaboration of all the neurological departments of the area. Several concurrent sources of cases are used. Patients are prospectively followed-up from diagnosis. Survival is calculated with life tables.

Results: In the 10-year period of observation, a total of 1259 new cases of ALS were detected, 687 men and 572 women. The mean age at onset was 64.9 years (SD 11.2) (mean 64.6 (SD 10.1); women, 65.4 (SD 10.7); p = n.s.). The mean time delay from onset to diagnosis was 10.4 months (SD 10.1), with a significant decrease in the last 5-year period (1995–1999, 11.0 months; 2000–2004, 9.7 months; $p = 0.001$). The mean annual crude incidence rate in the period 1995–2004 was 2.90 (95% c.i., 2.72 to 3.09). The lifetime risk of developing ALS is 1/278 among men and 1/431 among women. Incidence rates were significantly higher in men than in women. The incidence rates did not change when comparing the first 5 years (1995–1999, 2.87/100,000) and the second five years (2000–2004, 3.00/100,000) of the study. A total of 319 patients were alive at the prevalence day (December 31st, 2004), corresponding to a crude prevalence rate of 7.4 (95% c.i., 6.6–8.3)/100,000 population. There was a slight but not significant increase of median survival time (1995–1999, 915 days; 2000–2004, 1005 days). There was a significant increase of the number of patients who underwent endoscopic nutrition (PEG) (18.1% vs. 29.2%; $p = 0.0001$), and non-invasive ventilation (NIV) (8.9% vs. 16.7%; $p = 0.0001$), whereas the number of patients who performed tracheotomy only slightly modified (8.3% vs. 10.6%).

Discussion: ALS incidence did not change during the 10-year period of observation in this population-based on the prospective register. The significant reduction of the mean time delay is likely to indicate an increased awareness of ALS among the neurologists and other physicians in the examined area. Despite the increased use of PEG and NIV, there was only a modest increase of median survival.

P77 THE AUSTRALIAN MND DNA BANK: A CONTINENT-WIDE GENE-ENVIRONMENT RESOURCE

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Keywords: DNA bank, association study, gene-environment interaction

Background: Current thinking is that sporadic MND (SMND) is a complex disorder most likely due to a gene-environment interaction. Large numbers of cases and controls are needed for genetic association studies to provide sufficient power for reproducible results. In addition, environmental exposures or demographic factors may be risk factors for SMND. Until recently no DNA collections of SMND were available in the southern hemisphere. We therefore set up an Australia-wide DNA and environmental data resource for MND researchers.

Objectives: (1) To collect blood DNA samples and environmental data from sufficient numbers of people with SMND and controls to perform gene-environment studies. (2) To offer this material to both Australian and international researchers.

Methods: Since 2000, blood samples have been collected from donors throughout Australia, both in large population centres and in remote country areas. Donors fill out 8-page questionnaires on demographic and environmental factors. Clinical information on MND donors is received from treating neurologists.

Results: The Bank currently has 1,830 blood DNA samples with matching questionnaires. Of these, 32% have SMND, 24% have SALS, and 63% are controls. The proportion of

donors from each state is: New South Wales 51%, South Australia 12%, Queensland 12%, Victoria 10%, West Australia 7%, ACT 5% and Tasmania 2%. The great majority of donors are Caucasian in origin. Only 4 samples are from indigenous Australians. Samples from the Bank have been used in a number of SMND genetic studies (1–5).

Discussion: This is the first attempt to collect DNA samples from people with SMND throughout a whole continent. We estimate that we collect each year about 30% of all cases of SMND in Australia. Strategies are in place to collect more samples from remote areas. The Bank could be particularly useful for replicating genetic or environmental studies with a southern hemisphere population. Further requests for samples are anticipated as the number of donors increases to our target of 1,000 SMND patients and 2,000 controls.

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P78 GENOME-WIDE STUDY OF COPY NUMBER VARIATION IN THE IRISH AND DUTCH ALS POPULATIONS

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Keywords: CNV,genome wide study,genetics

Background: Sporadic ALS is an unrelenting neurodegenerative condition characterized by progressive limb and bulbar weakness. Genetic risk factors have been implicated in its pathogenesis. Copy number variations (CNVs) have the capacity to alter gene dosage and CNVs in SMN are a precedent for a biological role for CNVs in ALS.

Objective: To investigate the contribution of CNVs to ALS in the Irish and Dutch populations.

Methods: We did a genome-wide analysis of CNVs by comparison of Illumina 550K SNP array data on 206 Irish patients with ALS and 202 Irish controls with Illumina 317K SNP array data on 445 Dutch patients with ALS and 423 Dutch controls. The total number and length of CNVs, association of common CNVs with ALS and the co-occurrence of novel ALS-specific CNVs was examined using the QuantiSNP algorithm and data from the Database of Genomic Variants build 36.

Results: We detected 4,987 CNVs using 550K data in 408 Irish individuals and 4,103 CNVs using 317K data in 868 Dutch individuals. Using pooled data, no SNP showed a significant copy number difference between ALS patients and controls after correction for multiple testing. We identified 16 genes bearing loss or gain of copy number exclusively among ALS patients replicating in both the Irish and Dutch analyses. Among these, there were three genes where the same copy number alteration was observed in 6 individual patients. These variants did not occur in controls and have not been reported in previous studies of CNVs.

Discussion: Common CNVs in the regions of the genome included on the current SNP arrays do not alter susceptibility to ALS. However, rare CNVs observed uniquely in ALS patients may contribute to ALS pathogenesis. Future work should seek to profile the contribution of CNVs located in regions not covered on the present SNP platforms.

P79 A GENETICAL GENOMICS APPROACH TO IDENTIFY CAUSAL GENE NETWORKS IN ALS

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Keywords: genomics, networks, susceptibility

Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, characterized by the selective death of central and peripheral motor neurons. Mulitple genetic risk factors are considered to be implicated in disease susceptibility.

Genome wide studies that are aimed at identifying this genetic susceptibility typically involve arrays with many expression probes or genotype probes. Also in ALS, a number of genome-wide association studies have been performed. Due to the overwhelming number of hypotheses in relation to sample sizes, these studies are most often underpowered, while biologically relevant markers and probes are present in the final results (type II errors).

It has been shown that a large part of the expression of mRNA is under genetic control, also in humans. If a genetic marker is in or near an expression probe, the genetic effect is considered to be in "cis" with the probe, e.g. due to a promoter polymorphism that drives expression. If the marker is more distant from the probe, the genetic effect is considered to be in "trans" with the probe(s). The combined analysis of genotypic data and expression data in order to elucidate the genetic determinants of mRNA expression is known as "genetical genomics".

Objective: To identify genetically determined differential mRNA expression in ALS versus healthy controls (cis-effects), and to identify gene networks that are implicated in ALS susceptibility (trans-effects).

Methods: 217 subjects, 108 patients with ALS, and 107 age-, and sex matched controls were included. These subjects were genotyped using the 317K Illumina Beadarray SNP chip, in combination with the Illumina human-8 refseq V1 and V2 expression chips. After quantile normalization and log transformation, we used a variance components approach with multivariate linear regression to determine the associations between SNPs and expression probes, correcting for batch effects, sex, and age. In addition ALS specific SNPs that were associated with expression probes (eSNPs) were identified by also including an interaction term between genotype and group status (ALS or control).

Results: After quality control of the SNP markers, 305837 SNPs were included (out of 317503), and 15,123 expression probes, that were shared between the V1 and V2 arrays and that had a >95% unique BLAT hit in the current reference genome. Using a false discovery rate of 0.05, we identified 580

cis eSNPs, and 250 trans eSNPs, with several eSNPs showing a relationship with multiple expression probes, corresponding to potential gene networks. We will determine which cis eSNPs are determinants of the differential mRNA expression, and which trans eSNPs are specific to ALS.

Discussion: Our preliminary results show that the combined analysis of genetic markers and expression probes is able to identify which differential mRNAs are causal, rather than reactive to ALS. In addition, the identified ALS specific gene networks are interesting candidates for further (functional) investigations.

P80 GENETIC POLYMORPHISMS IN ALS REVISITED: A SYSTEMS BIOLOGY APPROACH *IN SILICO*

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Keywords: Genetics, SNPs, Systems Biology

Background: Amyotrophic lateral sclerosis (ALS) is a neurological disorder characterized by the progressive degeneration of motor neurons, muscle atrophy and eventually death. There is currently no cure for ALS. The etiology of ALS is not well understood and it is unclear how genetic regulations responsive to environmental factors might affect the irreversible and progressive loss of motor neurons.

Objectives: A growing number of genes with single nucleotide polymorphisms (SNPs) or mutations have been implicated in ALS in recent literature. In order to better characterize molecular pathways, critical genetic factors and predictive patterns in ALS, we conducted a comprehensive systems biology analysis using the Pathway Studio^{TM} program.

Methods: Pubmed scans using search terms in Pathway Studio^{TM} automatically extracting gene names from abstracts were used to create pathway maps connecting proteins, complexes, small molecules and other entities such as diseases and cell processes. These maps included genes with or without known ALS-related polymorphisms (ALS-SNP genes). In parallel, a list of ALS-SNP genes was manually constructed using full text articles found in the literature presenting such genes listed in tables or text. Molecular networks identifying direct or indirect interactions/regulations between ALS-SNP genes were established. Published data indicating SNP association strength with ALS were integrated within these networks. Common targets and regulators were identified and initial networks created by Pubmed scans were used for cross-validation and refinements.

Results: Five pathway maps were obtained using the search terms "Amyotrophic lateral sclerosis" alone and with "polymorphism", "SNP", "mutation", or "gene" which consisted of 593 proteins/2368 regulations, 52/114, 13/16, 174/527, and 278/912, respectively. These maps included ALS-SNP genes and interacting genes with no known ALS-SNP. The list of ALS-SNP genes based on full text articles contained 85 genes. The minimal molecular network with most direct interactions included 115 proteins, 2,027 regulations, 26 cellular processes, 10 functional classes and 18 disease entities. Gene ontology groups with $p < 0.0001$ for this network included 1) angiogenesis, 2) synaptic transmission, 3) response to hypoxia, 4) heme binding, 5) superoxide release, 6) response to oxidative stress, 7) potassium channel activity, 8) viral genome replication, etc. Each ontology group corresponds to a submolecular network containing ALS-SNPs gene(s) that may significantly favor or inhibit these processes.

Conclusion: Using Pathway Studio^{TM} program we were able to highlight diverse regulations potentially affecting or affected by ALS-SNP genes. Our approach identified closely interconnected pathways involving many ALS-SNP genes that can be influenced by environmental factors or be controlled by tissue-specific regulations. Functional analysis of these pathways is critical for the development of new drugs and molecular-based early diagnostic methods for ALS. In conclusion, systems biology tools significantly enhance the power of analysis of genetic regulations with etiologic significance in ALS.

P81 ALS IN THE OFFSPRING OF A CONJUGAL ALS CASE

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Key words: conjugal ALS, genetics

Background: Conjugal ALS cases have been described for more than 20 years. As it appeared important to determine whether the incidence of ALS in the children of those patients was higher than normally expected, we followed the offspring of 9 couples with ALS.

Objectives: To describe a family with ALS in both parents and in which a son recently also developed ALS.

Material and Methods: The couple were described in our work published in Arch Neurol (Corcia et al., 2003, family 3). The woman had bulbar ALS in 1984 at age 60 and died 18 months later. The man had flail arm syndrome since 1997 at age 72, he died 48 months later. This couple had 4 children, 2 sons and 2 daughters.

Results: The oldest child, a man, was first seen in our center in October 2005. He complained of cramps since 2004 and noted a right foot drop since April 2005. ALS was diagnosed due to diffuse fasciculations, weakness of the right leg and diffuse and active denervation in the four limbs and the abdomen. His ALSFRS was rated 45/48 at that time.

Differential diagnoses were ruled out with MRI of the brain, comprehensive biological examinations and lumbar puncture. Sequencing of the 5 exons of the SOD1 gene was normal.

He significantly deteriorated in the following months with (in February 2008) severe tetraparesis with amyotrophy and fasciculations and pyramidal syndrome in the 4 limbs. He was treated with riluzole since 2005 and has been received lithium for one month. His ALSFRS is now rated 32/48. Further familial investigations did not find any other ALS case in the family.

Discussion: To our knowledge, this is the first description of an ALS case in the child of a couple suffering from conjugal ALS. SOD1 mutations were absent. The three patients had an onset of different sites. All had, at one time in the course of their disease, both upper and lower motor neuron signs. It is not possible to draw any conclusion regarding the genetic implications of such a case. It nevertheless seems important to follow up the offspring of the conjugal ALS cases.

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P82 A MULTIPLEX ALS FAMILY WITH AUTOSOMAL DOMINANT D90A MUTATION

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Background: One of the most frequent SOD1 mutations in Europe is the homozygous D90A, of recessive influence. Despite some descriptions of ALS cases with heterozygous D90A, it has not yet been demonstrated that dominant cases may exist.

Objectives: To describe a multiplex family with heterozygous D90A mutation transmitted as a dominant trait.

Material and Methods: Samples from a family from Clermont-Ferrand were collected and the SOD1 gene sequenced for all exons in one patient and 11 other family members.

Results: This family is composed of 3 ALS cases. The proband had lower limb onset by the age of 62 in 2001 and is still alive. He had unequivocal upper and lower motor neuron signs. His father died of ALS in 1970 at the age of 71. He had lower limb onset and disease duration was 10 years. The proband's sister died of ALS at the age of 52. She had lower limb onset with a rapid bulbar involvement inducing death after 3 years of disease.

Results from SOD1 sequencing are featured on the pedigree. Our proband carried het-D90A mutation. This mutation was also present in 2 children of his sister and in one child of a brother who died of myocardic infarction at 62 years old. The father had one brother. This 91 years old man also carries het-D90A but does not complain of neurologic troubles.

Discussion: In this family het-D90A is present or deduced by pedigree analysis, in all 3 ALS patients. One subject did not develop ALS, even after 90 years. One brother carrying the mutation died at 62 yrs old of another cause.

As in the hom D90A cases, all the patients had lower limb onset. This family comes from the same area as another family with compound heterozygote D90A/D96N already published. This compound heterozygote mutation was ruled out here.

As far as we know, this pedigree is the first to be described in which the distribution of the D90A mutation is undoubtedly autosomal dominant. However, penetrance is incomplete.

P83 SOD1 IN SPORADIC AND FAMILIAL ALS IN THE NETHERLANDS

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Keywords: SOD1, D90A

Background: *SOD1* is mutated in about a quarter of families with ALS in the US, the UK, Germany, Sweden, and Belgium. In most western countries *SOD1* mutations can also be found in approximately 5% of sporadic ALS patients. However, *SOD1* mutations appear to be very rare in families with ALS in Portugal, Switzerland and The Netherlands.

Objectives: To determine the prevalence of *SOD1* mutations in a Dutch population of familial and sporadic ALS patients.

Methods: 490 sporadic and 52 familial ALS patients were screened for mutation in *SOD1*.

Results: 2 mutations were identified in 2 patients with sporadic ALS; a homozygous D90A mutation and one novel mutation I99V. One mutation was identified in a familial patient; heterozygous D90A. In our population 0.004% of SALS patients and 1.9% of familial patients carried a *SOD1* mutation.

Discussion: Mutations in SOD1 appear to be rare in The Netherlands compared to most western countries. The finding of both a homozygous and a heterozygous D90A mutation is very interesting, considering D90A mutations behave recessively in Sweden, but cause autosomal dominant ALS in Belgium.

P84 THE E22G MUTATION IN THE CU/ZN SUPEROXIDE DISMUTASE GENE PREDICTS A LONG SURVIVAL TIME. CLINICAL AND GENETIC CHARACTERIZATION OF A SIX-GENERATION ALS1 SPANISH PEDIGREE

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Keywords: Q21G, SOD1 gene, clinical-genetic characterization

Background: Despite the genetic heterogeneity reported in familial ALS (fALS), SOD1 gene mutations are the most frequent cause of fALS, accounting for around 20% of familial cases (ALS1) and isolated sporadic cases. Some mutations are associated with a long survival time, while others are linked to a very rapid progression. For this reason, clinical-genetic characterization of ALS1 families is important as it can provide information on the phenotype associated with a given mutation, the distribution of SOD1 mutations in different ethnic groups, and can clarify the genotype-phenotype correlation in patients with SOD1 gene mutations.

Objectives: To describe the phenotype linked to this previously reported SOD1 gene mutation, E22G (E21G in the old nomenclature), in a large ALS1 Spanish kindred. This mutation was previously reported in a sporadic ALS case and in a Canadian ALS family but no clinical information was available or reported.

Methods: Clinical characterization including gender, age at onset, site of onset and survival, were available from twelve affected members, belonging to a six-generation pedigree. The possibility of gender predominance or anticipation was also analyzed. DNA samples were available in three living symptomatic members. Informed consent for blood samples was obtained. Genomic DNA was extracted from whole blood lymphocytes using a QIAGEN\DNA blood midi-kit (QIAGEN, CA, USA). PCR fragments encompassing the entire SOD1 gene were amplified from the total DNA using 5 sets of

20-mer primers. Sequence analysis of these fragments was performed with an ABI Prism 310 automatic sequencer.

Results: An A-to-G transition at nucleotide position 65 (c.65A >G) leading to an E22G residue change was identified in the three affected ALS patients. The phenotype was similar in all affected members in our E22G family. Initial symptoms occurred in the distal limb muscles, predominantly in the legs, and there was a mean survival time of approximately 13.3 years. Mean age at onset was 50.6 years (SD 8.2). The prevalence in males and females was similar, with no difference in phenotype as regards gender. The age range for onset of symptoms was between 40 and 69 years of age, although 66% of the members presented symptoms before their fiftieth birthday. The information available for five affected parent/affected offspring pairs suggested no apparent anticipation, as has been described in some SOD1 mutations.

Conclusions:. E22G is the ninth SOD1 gene mutation reported in Spain, and the third of these associated with long survival (G38R- previously G37R, and D77V- previously D76V). Our results emphasize the importance of genetic and clinical characterization of ALS1 families around the world in understanding the genotype-phenotype relationships of each SOD1 mutant and their relative frequency in different ethnic groups worldwide.

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P85 RESPIRATORY ONSET IN AN ALS FAMILY WITH L144F SOD1 MUTATION

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Key words: Familial ALS, respiratory onset, SOD1 mutation

Background: ALS cases with SOD1 mutations may sometimes present with unsual clinical features such as an absence of upper motor neuron signs (e.g. A4V, G93C).

Objectives: To describe a family with initial and predominant respiratory involvement in which an L144F SOD1 mutation was uncovered.

Results: A man was refered to our center in August 2007, at 55 years old, showing symptoms of intense fatigue and dyspnea since January 2007. At that time, he experienced difficulties breathing in a lying position. His clinical state required non invasive ventilation (NIV) from July 2007. Upper and lower motor neuron signs were noted on the right upper and lower limbs with bisk tendon reflexes, fasciculations and amyotrophy. Denervation was noted at EMG on all four limbs. ALSFRS was 32/48. In January 2008, he was clearly deteriorating with an ALSFRS rated 22/48.

The mother of this patient died at 83 years old in 2004. She had dyspnea since 1990 and NIV began in 1994. In 2000, she was well adapted to NIV and in all limbs weakness was present. She had to walk with a support. Weight loss was noted but not quantified. In 2002, hypercapnia was noted (pCO2 50mmHg) despite ventilation.

The maternal uncle of our patient died several years before at the age of 70. He had initial respiratory deficiency and diffuse amyotrophy. He lived in Yugoslavia.

Discussion: L114F SOD1 mutation was initially described by Deng et al in 1993. While atypical clinical features are not exceptional in ALS families with SOD1 mutations, to our knowledge this is the first family with a respiratory onset in the three affected members. Given this atypical onset, diagnosis was not ascertained until death in the mother and the maternal uncle. While a significant number of patients with SOD1 mutations have limb and particularly lower limb onset, our family underlines the fact that a respiratory onset in an ALS patient should not exclude the possibility of either both a familial case or a SOD1 mutation.

P86 HIGH RESOLUTION MELTING (HRM) ANALYSIS FOR MUTATION SCREENING OF THE CU/ZN SUPEROXIDE DISMUTASE (*SOD1*) GENE

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Keywords: High resolution melting (HRM) analysis, SOD1 gene

Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder selectively affecting motor neurons in the spinal cord, brain stem and cerebral cortex. Mutations in the Cu/Zn superoxide dismutase (*SOD1*) gene are the most frequent genetic defects known to underlie ALS, accounting for 20% of familial cases and 2 to 4% of apparently sporadic ALS (SALS). We tried to use high resolution melting (HRM) analysis as a rapid and easy tool to screen mutations in the *SOD1* gene.

Objective: To screen mutations in the *SOD1* gene we performed HRM analysis. Also, we applied this method to examine the prevalence of *SOD1* mutations in Japanese SALS cases.

Method: With informed consent, genomic DNA was extracted from lymphocytes using standard procedures. We designed PCR primers for HRM analysis to screen all 5 exons of the *SOD1* gene. First, we examined 19 known *SOD1* mutations to determine the sensitivity of this method. Then we used this method for approximately 190 Japanese SALS cases who fulfilled the revised El Escorial criteria.

Results: HRM analysis could clearly distinguish 18 of 19 previously identified mutations in normal controls. Three different *SOD1* mutations were found in the SALS cases. The mutations identified were Cys6Tyr (C6Y) and Gln22His (Q22H) in exon 1, and Ser134Thr (S134T) in exon 5.

Discussion and Conclusion: The prevalence of *SOD1* mutations among Japanese SALS cases was 1.6%. HRM analysis is a sensitive method to screen mutations in the *SOD1* gene.

P87 SCREENING OF SUPEROXIDE DISMUTASE 1 GENE MUTATION AND SNP GENOTYPING BY HIGH-RESOLUTION MELTING IN CHINESE AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Keywords: Amyotrophic lateral sclerosis, single nucleotide polymorphisms, Chinese population

Background: Approximately 20% of FALS cases and 2% of overall ALS cases have more than 130 different identifiable mutations located in all five exons of Cu/Zn superoxide

dismutase (SOD1) gene worldwide. We have reported our screening results of SOD1 gene mutations which identified by DNA sequencing in sporadic cases with five mutation found in 111 cases and 19 individuals from three ALS family, but routine screening for SOD1 mutation using DNA sequencing is expensive, more economic procedure needed. Sporadic ALS may be caused by the interaction of multiple environmental factors and previously unknown genes. A number of association studies have been performed in an effort to find genetic component, several specific SNPs seem to be strongly associated with susceptibility to SALS in different populations of European and American ancestry. To assess the role of those genetic variations in Chinese ALS, we performed a SNP genotyping.

Objectives: We developed a procedure for screening of SOD1 gene mutations and SNP genotyping using a PCR and high resolution melting analysis method.

Methods: The El Escorial ALS diagnostic criteria were used. Genomic DNA was prepared from ALS patients and health controls using standard procedures. PCR amplification of five exons of the SOD1 gene was performed using primers as previously described. Additional samples with known mutations were used as positive controls. Asymmetric PCR amplification of DPP6 and FLJ10986 was performed using self designed primers. After PCR, melting curves were generated by monitoring the fluorescence of a saturating dye on LightScanner. Statistical analysis was performed comparing the ALS and Control groups.

Results: A V47A mutation was found in exon 2 of SOD1, which was confirmed by DNA sequencing, with a different phenotype compared with the same allele locus mutation reported (V47F). We found a variant on 8,227 base of SOD1, shown to be a SNP. None of the suggested associations (DPP6 and FLJ10986) were found in Chinese ALS patients or healthy controls.

Discussion and Conclusions: This study reports a simple and economic procedure for screening SOD1 mutations and SNP genotyping in Chinese ALS patients. Identification of the V47A mutation increases the number of SOD1 mutation to 6 in Chinese ALS SOD1 mutation screening series. High resolution melting analysis shows excellent accordance with identified by DNA sequencing in the samples with some mutations reported as previously (V29A, H46R, N86 I, G72C and E133V). SNP variation in DPP6 and FLJ10986 failed to show a major effect on susceptibility to Chinese sporadic ALS in this study. Further confirmation is warranted in additional large sample size of the Chinese population.

P88 INVESTIGATION OF FAMILIAL ALS IN CHINA BASED ON A COUNTRY-WIDE DATABASE

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Keywords: Chinese, pedigree, copper/zinc superoxide dismutase 1

Background and Objectives: Familial amyotrophic lateral sclerosis (FALS) is reported in approximately 5%-10% of ALS in the world. Inheritance is usually autosomal dominant with variable penetrance. Mutations in copper/zinc superoxide dismutase 1 (SOD1) are found in approximately 20% of FALS. As a huge country with a big population, however, China has no data of FALS so far. In this study, we try to investigate the number of Chinese patients with FALS based on our country-wide database.

Methods: Data from ALS patients included in the ALS Clinic database of Peking University Third Hospital from January 2005 to end of March 2008 were included. The diagnosis of ALS follows the El Escorial criteria (revised version). The geographical distribution of patients covers more than 93% of Chinese administrative provinces (excluding Taiwan and Tibet). Some of these families have been screened for SOD1 mutations.

Results: During the study period, a total of 749 individuals were diagnosed with probable or definite ALS. Among them, 11 pedigree patients from 10 different provinces all over the country were found to have a positive family history of ALS, with the bulbar-onset form accounting for 9.1% (1/11), and the rest 90.9% limb-onset form (10/11). Seven family pedigrees included from two to five patients with ALS (63.6%), while four pedigrees had more than 12 affected individuals (36.4%). Two of these families had pedigrees of more than 20 patients in 6 generations, with fully penetrant autosomal dominant inheritance (18.2%). The total FALS patients from 11 family pedigrees were 84 (accounting for 11.2% in our ALS database, 84/749), and the male-to-female ratio was 0.79 (37/47), with a median age of onset of 48.6 years. Following their diagnosis, a median lifespan was 5.2 years, ranging from 1 year to longer than 15 years. Most FALS patients show autosomal dominant inheritance (90.9%) except for one with autosomal recessive inheritance (9.1%). SOD1 analysis was performed in 5 of 11 FALS family pedigrees, and only two families (40%) were identified SOD1 mutations, in which one is a rare mutation Ser105Leu in exon 4, and another is a novel mutation Glu133Val in exon 5, respectively, both with quite different from those previously described.

Conclusions: In this study, we found the percentage of FALS patients is 11.2% in our country-wide database and these 11 families come from 10 Chinese administrative provinces. The survival of FALS cases seems longer, but the form of bulbar-onset and the difference of gender are lower than reported. The incidence and pattern of inheritance are similar to the data reported in the literatures. Our data is the first report of FALS in China, which will be important and helpful for researchers on ALS genetics.

P89 A CHROMOSOME 21-LINKED FAMILY WITH ALS BUT NO MUTATIONS IN THE SOD1 GENE

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Keywords: SOD1, linkage analysis, mutation detection

Background: Mutations in the SOD1 gene have been well-established as a cause of approximately 15–20 percent of cases of familial ALS. We have identified a multigenerational family with ALS from France. No mutations were identified in the SOD1 gene after sequencing the complete gene in five affected individuals with ALS.

Objectives: Our objective is to determine the genetic cause for ALS in this family.

Methods: DNA from seven individuals (three affected, four unaffected) was sent for a 550-marker eight centiMorgan whole genome scan. This was followed by PCR amplification of the SOD1 gene using various sets of primers.

Results: The genome scan yielded only one locus with a LOD score above 1.5, specifically a region on chromosome 21

between markers D21S1432 and D21S266. This region contains the *SOD1* gene. A follow-up PCR analysis was conducted with a second set of non-overlapping primers for SOD1 which spans the coding and UTR regions. In addition, a long-range PCR strategy was employed to test for the possibility that intronic insertions or deletions may be present. In both cases, no causative variants were detected.

Discussion: Several possibilities exist as to why no mutations were identified. A mutation may exist in a regulatory region, or deep within an intron. Alternatively, a larger and undetected copy number variant may be present at the *SOD1* locus. Finally, another nearby gene which is causative for ALS may be mutated in this family. It was the examination of this last scenario for the MAPT locus on chromosome 17 which led to the discovery that mutations in the progranulin gene cause frontotemporal dementia. Thus, the identification of the mutation that is responsible for ALS in this family will enable an understanding of further genetic mechanisms for developing ALS and may help determine the genetic cause of ALS for a proportion of familial ALS patients.

P90 CHARACTERISATION OF THE PROPERTIES OF A PUTATIVE NEW DISEASE ASSOCIATED FAMILIAL ALS LOCUS ON CHROMOSOME 12: D-AMINO ACID OXIDASE

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Keywords: molecular genetics, amino acid metabolism, neurogenetics.

Background: Following the discovery of SOD1 mutations in familial Amyotrophic Lateral Sclerosis (FALS), two further genes have been identified causing classical ALS, VAPB and TDP-43. However, these three genes alone can account for no more than 30% of cases and the remaining causal genes remain to be elucidated.

Objectives: To carry out a genome screen using microsatellite markers in extended kindreds in order to identify potential new FALS loci

Methods: A genome screen was carried out in extended FALS kindreds which identified a 9.2 cM region on chromosome 12 linked to disease in a single family. A disease-associated mutation was detected following sequencing of candidate genes in this region and N-terminal GFP fusion constructs were generated and expressed in COS-7 and neuroblastoma (N2a) cell lines to evaluate the effect of this mutation.

Results: A putative locus was indicated on chromosome 12q22–23 from a maximum multipoint LOD score of +3.33 at D12S1646 localising the region to a 9.2 cM interval between the markers D12S330 and D12S79. This region was screened for mutations in candidate genes and a point mutation, R199W, in the open reading frame of the D amino acid oxidase (DAO) gene was demonstrated in all affected individuals in this extended family. We screened for the R199W mutation in a total of 439 unrelated individuals (277 controls, 23 sporadic ALS cases and 139 FALS index cases, all of Caucasian origin residing in the UK) in order to determine its prevalence in the general population but no further individuals carried this mutation. To obtain insight into the effect of the R199W mutation, the DAO transcript and enzyme activity were examined in post mortem spinal cord and motor cortex tissue available from an individual with the R199W mutation and compared to control and sporadic ALS cases. The expression of GFP fusion DAO in COS-7 cells (R199W DAO and wild-type DAO) yielded abundant levels of protein (~39kDa) corresponding to DAO, that localises appropriately to peroxisomes. Enzyme activity was severely impaired in the mutant form, obtained both from cell extracts and the autopsy case from the affected pedigree

Discussion and Conclusion: This is the first report of an ALS-associated mutation in DAO in FALS. DAO is abundant in spinal cord where it has been demonstrated to regulate the major excitatory neurotransmitter, glutamate, through its action on D-serine. DAO also has an important role in the inactivation of toxic D-amino acids, which accumulate in the central nervous system with age. A mutation at the active site of DAO is likely to lead to pathological consequences which could underlie ALS pathogenesis through the potentiation of glutamate-mediated neurotoxicity. Importantly, D-serine is now known to accumulate in the spinal cord in ALS and in the SOD1 mouse model of ALS indicating that this abnormality may represent a fundamental component of the disease process. Our long-term aim is to establish whether this DAO mutation is causal in ALS and whether modifying D-serine levels affects disease progression.

P91 MONOAMINE OXIDASE B GENE POLYMORPHISM IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: MAOB, Polymorphism, Association

Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease affecting 1–3 in 100,000 worldwide. Its prevalence is somewhat more in men, and has an average onset age of 56 years, with onset ranging from 20 to 80 years. ALS is a sporadic disease with 10% familial. Death occurs within 3 to 5 years after the first symptoms from respiratory failure.

Monoamine oxidase B (MAO B) is a mitochondrial outer membrane flavoenzyme involved in the degradation of biogenic amines, and is a well-known target for anti-depressant and neuroprotective drugs. MAO B levels induce apoptosis in neuronal and kidney cells. Elevated MAO B levels have been also reported in plaque-associated astrocytes of brains from Alzheimer's patients. MAO B may also be important in pathological processes resulting from exposure to various xenobiological compounds. MPTP is biologically activated by MAO B which causes chemically induced Parkinson's disease. Components in tobacco smoke inhibit MAO B gene expression. The MAO B gene is located on Xp11.23, and the

protein contains 520 amino acids. The crystal structure of MAO B reveals that it is dimeric.

Objectives: The fact that MAO B may play a role in the pathogenesis of neurodegenerative disorders, has been based on the assumption that this enzyme is responsible for neuron oxidative damage through the production of free radicals. We analysed a single base difference (A or G) in intron 13 of the MAO B gene in connection with sporadic amyotrophic lateral sclerosis (SALS).

Methods: We used a PCR-RFLP method to analyse the genotypes and SPSS to do the statistics.

Results: We studied 248 SALS patients (age range 48.40 ± 16.840) and 116 (age range 52.67 ± 15.522) controls. In this cohort study, the MAO B gene polymorphism was not associated with SALS (Chi2 = 2.231; P = 0.328). The distribution of the MAO B AA, AG, and GG genotypes was 44.0%, 19.0%, and 37.1% in controls and 50.8%, 19.8%, and 29.4% in cases respectively. The distribution of the MAO B A allele was 53.45% in controls and 60.69% in cases.

Conclusion: In conclusion, the MAO B intron 13 AG polymorphism was not associated with SALS. Therefore it is unlikely to play a role in the etiology of SALS.

P92 COMT VAL158MET POLYMORPHISM AND SUCCEPTIBILITY TO AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: COMT gene; Polymorphism; Association

Background: Amyotrophic lateral sclerosis (ALS) is a multifactorial neurogenetic disorder characterized by the gene interactions and environmental factors. Epidemiologic studies on ALS reveal that the prevelance of ALS is 1 to 3 in 100,000 worldwide. The mean ALS onset age is approximately 56.3 ± 11.1 years, with no gender difference.

Catechol-O-methyltransferase (COMT) is a ubiquitous enzyme that catalyzes the O- methylation of catechols and plays a role in the metabolism of neurotransmitters such as dopamine, noradrenaline and adrenaline. The COMT gene, located on chromosome 22q11, encodes both soluble and membrane-bound forms of this enzyme. A G-to-A transition at codon 158 of the COMT gene, resulting in the substitution of methionine for valine, is designated the L (low activity) allele, in contrast to the H (high activity) allele.

Objective: The aim of the study was to determine whether the COMT Val158Met polymorphism was associated with ALS.

Methods: A PCR-RFLP method was used in analysing the genotypes. Statistics were calculated using the SPSS v 12.

Results: We studied 124 ALS patiens and 124 controls. The distribution of the COMT 158 H allele was 56.85% in cases and 52.42% in controls. The distribution of the COMT 158L allele was 43.15% in cases and 47.58% in controls.

Conclusions: The COMT Val158Met polymorphism was not associated with ALS ($\chi^2 = 2.606$; P = 0.272). It seems that the COMT Val158Met polymorphism does not play a role in the etiopathogenesis of ALS.

P93 GENETIC SCREENING OF THE NRF2 ANTIOXIDANT PATHWAY IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Keywords: NRF2, Antioxidant pathway, Genetic screening

Background: Oxidative stress has been implicated in the pathogenesis of amyotrophic lateral sclerosis (ALS) and other neurodegenerative diseases. Antioxidant response elements (AREs) are transcription enhancers located in the promoters of antioxidant defence and phase II detoxification genes. ARE-containing genes are up-regulated via the redox-sensitive transcription activator nuclear factor erythroid-2 related factor 2 (NRF2). Under conditions of oxidative stress this protein translocates to the nucleus where it activates the transcription of ARE-containing genes. There is accumulating evidence to suggest that the NRF2/ARE pathway is disrupted in ALS. The expression of *NRF2* and ARE-containing genes is decreased in mutant SOD1 cell culture models and familial ALS spinal cord motor neurones (1,2,3). Increased ARE-driven gene transcription in astrocytes is, moreover, protective of co-cultured neuronal cells suggesting that modulation of the NRF2/ARE pathway could be a useful therapeutic strategy in ALS (4,5).

Objectives: To sequence genes from the NRF2/ARE pathway in ALS patients and controls in order to identify ALS associated genetic variants. The functional consequences of any disease associated genetic changes discovered will be investigated.

Methods: DNA was extracted from post-mortem brain tissue obtained from neuropathologically proven ALS patients and control subjects stored within the Sheffield (UK) brain tissue bank. Tissue was donated for the purpose of research and fully informed consent obtained. Exons of candidate genes were amplified using the polymerase chain reaction and sequenced using BigDye® terminator methodology. Sequence analysis was performed using Chromas and Sequencher™ software.

Results: To date we have screened the 5 exons and promoter region of NRF2, plus the protein coding exons of three ARE-containing genes in 126 ALS patients. A number of novel sequence variants have been identified. Those that change the amino acid sequence, and may therefore have a functional effect on the protein, have been prioritised for mutation screening in additional patient and control samples.

Discussion: This is the first genetic study to target the NRF2/ARE pathway in ALS patients. NRF2 is a master regulator of antioxidant defence. If function altering genetic changes are identified in ALS patients, this will provide further evidence of disrupted NRF2/ARE signalling in ALS and pave the way for further functional studies.

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P94 GENETIC VARIATION IN DPP6 GENE IS NOT ASSOCIATED WITH SUSCEPTIBILITY TO AMYOTROPHIC LATERAL SCLEROSIS IN TWO EUROPEAN POPULATIONS

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Keywords: amyotrophic lateral sclerosis, genome wide association, dipeptidyl-peptidase 6

Background: Only 15% of amyotrophic lateral sclerosis (ALS) patients have a family history, the remaining cases occurring sporadically. While familial ALS is well characterized with several causative genes identified to date, the genetics of sporadic ALS is poorly understood. Last year three independent genome-wide association (GWA) studies (1,2,3) found no single gene strongly associated with susceptibility to ALS; suggesting that a complex interaction between environmental factors and many susceptibility genes of small effect best describes this disease. One such susceptibility gene of small effect recently nominated by a GWA study from different populations of European origin is dipeptidyl-peptidase 6 (DPP6) which slightly increases the risk of developing ALS (OR 1.3) (4, 5).

Aims: To confirm the association of DPP6 with ALS phenotype we tested the candidate polymorphism rs10260404 in two other European populations, Italian and British.

Methods: The Italian cohort included 980 cases and 1119 healthy controls while the British cohort consisted of 500 cases and 500 controls. The Italian cohort tested in this study was collected by the Italian ALS Consortium created from the collaboration of 7 different institutions located in North Italy. We are recruiting more cases in other Italian neurological institutions for the first large-scale Italian SALS Screen. The British cohort was collected at King's College London. Individuals were genotyped following standard procedures (TaqMan, Applied Biosystems) and allelic discrimination was scored using an ABI799HT.

Results: Assuming an odds ratio of 1.3 with a causative allele frequency of 0.4, as described by the authors, we have >99% power to detect an association at $p = 0.05$. Preliminary data show no evidence of association with susceptibility to ALS (Fisher's exact chi-squared test $p = 0.9$). Genotype scores were tested for association with age at onset (AAO) on 700 cases of the Italian cohort and no significant association was detected.

Conclusion: The polymorphism rs10260404, as with other variants reported to be associated with sporadic ALS, was not replicated in a different population. These findings highlight the genetic heterogeneity of sporadic ALS even within European populations.

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P95 ASSOCIATION OF THE *H63D HFE* ALLELE WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: genetics, HFE, iron

Background: Five independent groups have published the frequencies of *HFE* polymorphisms in ALS patients compared to various control groups. A meta-analysis of these studies shows that possession of at least one *H63D HFE* allele is associated with a 26% increased risk of developing ALS.

Objective: To determine if the haplotype containing the H63D HFE allele is associated with ALS and determine the linkage of this haplotype with other haplotypes.

Methods: DNA samples were obtained from 115 ALS patients and 121 normal controls from a single outpatient Neurology clinic. DNA samples were also obtained for 557 ALS patients and 519 normal controls from the NINDS Neurogenetics Repository at the Coriell Institute for Medical Research. We genotyped for the *H63D* allele and surrounding SNPs on chromosome 6 between positions 25,783,000 and 26,414,000 spaced approximately every 10 kb by sequencing. Additionally, we determined the linkage of the *H63D HFE* with nearby haplotype blocks.

Results: In the current study, the *H63D HFE* allele was not significantly associated with ALS (heterozygotes: OR 0.90, 95% CI 0.70–1.17; homozygotes: OR 1.69, 95% CI 0.87–3.28). Combining our results with previous association studies shows a dose-dependent association of the H63D HFE allele with ALS (heterozygotes: OR 1.18, 95% CI 1.03–1.34; homozygotes: OR 2.04, 95% CI 1.48–2.80). The common region containing the *H63D* allele spanned 44.3 kb including the genes *HFE, HIST1H4C, HIST1H1T, HIST1H2BC, HIST1H2AC*, and was the same between cases and controls.

Discussion and Conclusions: While the results of our current study show no significant association of the *H63D HFE* allele with sporadic ALS, our study may have been underpowered to detect a significant association, particularly for *H63D* homozygotes. The combined analysis of all available data, however, shows a significant association of this allele with ALS in a dose-dependent fashion. The region containing the *H63D HFE* allele includes several highly conserved genes that increases confidence in the association between the *H63D* variant and ALS. Furthermore, the HFE gene variants are known to have potentially broad-ranging effects resulting from iron dyshomeostasis. The high degree of linkage disequilibrium in the region containing the *HFE* gene does not exclude the contribution of other genetic variants to the risk of sporadic ALS. The partial linkage disequilibrium between this region and other surrounding regions further extends the number of loci that may be associated with ALS. The data support the need for functional analysis of the *HFE* genetic

variants to contribute to our understanding of the roles of these variants to ALS pathogenesis.

P96 MUTATIONS OF THE ANGIOGENIN GENE IN FRENCH PATIENTS WITH SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: Genetics, Pathophysiology, Angiogenesis

Background: The pathophysiology of amyotrophic lateral sclerosis (ALS) remains unknown. Some factors that play a role in the angiogenesis have been linked to ALS. Several studies on the vascular endothelial growth factor gene (VEGF) suggested a link between angiogenesis and the pathogenesis of ALS. Recently, seven missense mutations in a second hypoxia responsive gene, the ANG gene, have been identified. The ANG gene located in 14q11.2 encodes angiogenin, a 14.1 kD enzyme which belongs to the pancreatic ribonuclease A superfamily. Angiogenin, whose expression is induced by hypoxia, mediates neovascularization and has been recently involved in neurite pathfinding. The characterization of new mutations in the ANG gene will certainly help to elucidate the molecular mechanisms linking ANG to ALS.

Objectives: To assess the frequency of ANG gene mutations in 855 French sporadic ALS patients.

Methods: We analyzed the coding region of the ANG gene in a large French cohort of 855 sporadic ALS patients.

Results: We observed a previously identified mutation (pI46V) in two ALS cases with no known family history, and found a novel mutation (pR121H) in one patient who developed ALS with rapid progression. We did not find an association between ALS patients and the rs11701 polymorphism, as previously reported in certain ALS populations of other ethnic origins.

Discussion and Conclusion: Overall, our findings support the implication of ANG gene mutations as a rare but widespread aetiology of ALS. The implication of the mutations in the ANG gene in ALS will have to be assessed by biological assays on angiogenesis and neurite pathfinding, and molecular assays on nuclear transport, RNAse activity, and rRNA synthesis.

P97 MUTATION SCREENING OF *ALS2*/ALSIN IN PATIENTS SUFFERING FROM PRIMARY LATERAL SCLEROSIS (PLS) AND UPPER MOTOR NEURON DOMINANT AMYOTROPHIC LATERAL SCLEROSIS (UMD-ALS)

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Keywords: Alsin,mutation,PLS

Background: PLS is a rare, sporadic, adult-onset variant of motor neuron disease (MND) in which upper motor neurons (UMN) giving rise to the pyramidal tract progressively degenerate. However, clinically there exists an overlap between PLS and Amyotrophic Lateral Sclerosis (ALS) and there is a debate as to whether PLS is a distinct disease entity or at one end of a spectrum of MND. Mutations in *ALS2* have been identified in a number of UMN disorders including Juvenile ALS, Juvenile PLS and Inherited Ascending Hereditary Spastic Paraperesis. Thus mutation screening of *ALS2* in clinically diagnosed PLS and upper motor neurone dominant (UMN-D) ALS patients may reveal mutations or polymorphisms associated with the disorder

Objectives: To screen the 34 exons of *ALS2* for mutations in a cohort of 95, clinically diagnosed PLS and UMN-D ALS patients.

Methods: 37 amplicons encoding the entire protein coding regions and intron/exon boundaries of *ALS2* were amplified and bi-directionally sequenced at a Core Genomics Sequencing Facility. Sequence chromatograms were analyzed using FinchTV (Geospiza) and aligned with Sequencher (Genecodes)

Results: Twenty three nucleotide substitutions spanning the entire *ALS2* gene have been identified. Out of these, 8 are previously unidentified variants. The variant c.1960A>G; pSer654Gly occurs at a highly conserved residue and the change of polar to hydrophobic amino acid may affect protein function. Variant c.1737+1A>G is predicted to abolish splicing of intron 7 and therefore likely to produce a mutant protein. Neither of these changes were found in 322 control chromosomes. The remaining 6 novel substitutions are situated in intronic sequences >50 bp from the nearest exon.

Conclusions: The *ALS2* was screened in a large cohort of adult onset PLS and UMN-D patients. Of the 8 previously unidentified variants, 2 are predicted to alter the Alsin protein and may therefore represent factors conferring susceptibility to UMN degeneration.

P98 NOVEL MUTATIONS OF SENATAXIN GENE ASSOCIATED WITH SPORADIC CASES WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: senataxin gene, mutation, sporadic amyotrophic lateral sclerosis

Background: Amyotrophic lateral sclerosis (ALS) is the commonest adult onset disorder of motor neurones, and among the most common of adult onset neurodegenerative diseases. Although enormous scientific progress has been made, its etiology and pathogenesis are still not well known, especially for sporadic ALS (SALS). FALS is caused by mutations of many genes including SOD1 (ALS1), ALSIN (ALS2), Senataxin (SETX, ALS4) and VAPB (ALS8). *SETX* is a related gene of ALS4. ALS4 is a rare autosomal dominant form of juvenile amyotrophic lateral sclerosis, characterized by distal muscle weakness and atrophy, normal sensation, and pyramidal signs. Patients with ALS4 usually have a juvenile onset of symptoms (ages <25 years), a slow rate of progression with a normal life span. SOD1 mutations are found in some apparently SALS patients, so hypothesised that *SETX* mutations may be associated with SALS patients.

Objectives: 45 patients with SALS from the Han ethnic group in South China and 120 healthy controls were studied.

Methods: All 21 exons of *SETX* including the intron-exon boundaries were amplified in the subjects using primer combinations, based on the intronic sequences of *SETX*. The full length of exon10 is 4,176bp, thus 10 pairs of primers were designed flanking this area. PCR products were generated using a GeneAmp PCR system 9700 (Applied

Biosystems, Foster City, CA, USA) using standard conditions. Amplified products were purified and subjected to direct sequencing. The sequences obtained were compared with genomic DNA sequence of *SETX* (NCBI Sequence Viewer NT_022184).

Results: All 21 exons of *SETX* were analyzed in 45 patients with SALS and 120 healthy controls. One missense mutation, one synonymous mutation and 19 novel polymorphisms were identified. The patients with mutations had different clinical phenotypes from ALS4.

Conclusions: Our study implied *SETX* may be associated with SALS as well as ALS4. Although the significance of the sequence variations is still unknown, the study broadened the mutation spectrum and polymorphism spectrum of *SETX* and expanded the clinical phenotype of *SETX* mutation. It might be productive to search in larger samples.

P99 INVESTIGATION OF *SMN* GENE IN ALS PATIENTS OF CHINESE ORIGIN

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Keywords: SMN1, SMN2, sporadic ALS

Background: Amyotrophic lateral sclerosis (ALS) is a rapidly progressive, incurable and terminal disease, whose pathogenesis is not clear. For years, some researchers reported that survival motor neuron (SMN) which is the determining gene of spinal muscular atrophy (SMA) is related to ALS, but there is no consistency of results. Moulard B *et al.* (1) considered *SMN2* deletion to be a risk factor for the specific subset of LMN ALS. Veldink JH *et al.* (2) reported *SMN2* deletion as a risk and poor prognostic factor for sporadic ALS. Corcia and colleagues (3) reported that abnormal *SMN1* gene copy number is risk factor for sporadic ALS. In 2005, Veldink JH *et al.* (4) further reported that lower *SMN2* copy number and lower level of estimated SMN protein increased susceptibility to and mortality rate of ALS. In 2006, Corcia P *et al.* (5) further reported that abnormal *SMN1* gene copy number is a genetic risk factor in sporadic ALS and there was no modulator effect of the *SMN2* gene. There are no related reports of the Asian population, especially of Chinese origin.

Objective: To investigate the information of *SMN1*/*SMN2* deletions and *SMN2* copy number in sporadic ALS patients and normal controls of Chinese origin, to investigate the risk factors for ALS in Chinese.

Methods: 61 patients with sporadic ALS and 60 healthy individuals of the Chinese Han population were screened for *SMN1* or *SMN2* deletions using PCR-RFLP, the detailed procedure for this has been described previously (6). All of the subjects were further investigated for the *SMN2* copy number by real-time fluorescence quantitative PCR which has also been described previously (7).

Results: No *SMN1* deletion has been detected in any of the ALS patients and healthy individuals. Two of the 60 healthy individuals were determined as having *SMN2* deletions and the frequency was 3.3%, but none of the ALS patients have *SMN2* deletions. The numbers of ALS patients who possess 1, 2, 3, 4 copies of *SMN2* are 13 (21.3%), 30(49.2%), 16(26.2%) and 2(3.3%) respectively, the numbers of healthy individuals who posses 1, 2, 3, 4 copies of *SMN2* are 18(30.5%), 25(41.7%), 12(19.4%) and 5(8.4%) respectively. Chi-square test comparing the *SMN2* copy number distribution between ALS patients and healthy individuals showed no significant difference ($p = 0.375$).

Conclusions: Our results don't show the correlations of *SMN1*/*SMN2* deletions and *SMN2* copy number to ALS. As the case number of this report is small, it is necessary to collect more Chinese ALS patients and carry out further studies in the future.

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P100 THE INITIAL GENE STUDY ON SPORADIC AND FAMILIAL JUVENILE MUSCULAR ATROPHY OF THE DISTAL UPPER LIMBS

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Keywords: Hirayama disease, Inheritance, X-chromosome

Background: Hirayama disease (HD) is also called juvenile non-progressive amyotrophy. Its mechanism is not clear. The disease is usually sporadic. Familial occurrence is occasional. There are only two cases in one pedigree in the reported pedigrees. Research on the molecular genetics of the disease has so far been restricted by the lack of a large pedigree.

Objective: A pedigree and sporadic cases of HD were used to investigate the role of SOD1 and SMN genes in the pathogenesis of the disease. To investigate the gene location of a Chinese HD pedgree.

Methods: Blood samples were collected from 14 sporadic HD patients and 15 members of the familial HD in which there are 6 patients in 3 generations. Polymerase chain reaction and single-strand conformation polymorphism of the SOD1 gene and PCR- restriction enzyme digestion analysis of exon 7 and exon 8 in SMN gene were scanned in 14 sporadic HD and in the patients in the pedigree of HD. DNA samples of the 15 members of familial HD were analyzed with 18 polymorphic microsatellites distributed on the X chromosome on the Human MapPairs marker set at 10cM interval by PCR. The linkage between eighteen markers on chromosome X and Hirayama disease was tested by MLINK software in the package of LINKAGE (version 5.1).

Results: The 5 exons of SOD1 gene were normal and the exon 7 and 8 gene deletion of SMN were not found in the familial or sporadic HD patients. Fifteen samples from the family with HD were genotyped using 18 polymorphic microsatellites which cover the whole X chromosome. None of the Polymorphic microsatellites suggested linkage and yielded a Lod scores which were less than 0 by means of the MLIN K software respectively.

Conclusion: SOD1 and SMN genes may not contribute to the pathogenesis of HD. The disease gene for this familial HD is not located on X chromosome, which suggests an autosomal dominant pattern of inheritance.

P101 HETEROZYGOUS S44L MISSENSE CHANGE OF THE SPASTIN GENE IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: spastin, missense S44L, ALS

Background: Amyotrophic lateral sclerosis (ALS) is a clinically and genetically heterogeneous group of disorders affecting the upper and lower motor neurons. There is increasing evidence that spastin is a candidate gene for ALS. Mutations in the spastin gene represent the most common cause of dominantly inherited hereditary spastic paraplegia (HSP). The majority of patients with spastin mutations develop uncomplicated HSP, but additional features are common.

Objectives: Here we report a patient with rapidly progressing adult-onset ALS. Molecular genetic analysis revealed a heterozygous missense change (S44L) of the spastin gene.

Methods: All 17 exons and flanking intronic sequences of the spastin gene were amplified by PCR and followed by single strand conformation analysis (SSCP) and direct DNA sequencing.

Results: Mutation analysis of the spastin gene identified a previously described heterozygous missense change (c.131C >L) located in exon 1 (RefSeq NM_014946). The sequence variant is predicted to replace serine by leucine in position 44 (S44L) of the putative spastin protein.

Conclusion: The single case of ALS with a spastin mutation presented here does not allow any conclusion about causality, but there is also no evidence against a monogenic cause of ALS by a spastin mutation. Alternatively, the spastin S44L change may serve as a previously unknown genetic risk factor for ALS. The present study suggests that spastin sequence changes may contribute to a wider range of phenotypes, including ALS. Given the common and perhaps universal finding of axonal dysfunction in both ALS and HSP, variants of the spastin gene may contribute to a common pathway in the complex pathogenesis of motor neuron disorders.

P102 SPG4 HEREDITARY SPASTIC PARAPLEGIA (HSP): THE INTRON 3 TAAT-DELETION IS UNLIKELY TO BE A PATHOGENIC MUTATION

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Keywords: hereditary spastic paraplegia, SPG4, mutation

The Spastic paraplegia type 4 (SPG4) is the most common cause of the hereditary spastic paraplegias (HSP) and accounts for approximately 40% of the dominantly inherited forms. More than 150 disease-related spastin (SPG4 gene)-mutations have been described so far. Missense, nonsense, frameshift and splice-site mutations as well as gross deletions have been reported in *SPG4*, all presumably acting via haploinsufficiency.

Here, we describe the genetic findings in three affected siblings (44–61 years of age) of a HSP-family. The deceased father also suffered from spastic paraplegia. All three siblings show pure HSP with slow progressive lower limb spasticity and urinary incontinence but without dorsal column disturbances and extraspinal involvement. Disease onset was from the late third to the early fifth decade. After approximately 20 years of disease, the ability to walk is preserved but reduced to less than 500 m with support.

We found the siblings to be compound heterozygous for a sequence variation within a 5 splice site of *SPG4* (1321+2T >G) and a previously described 4-base pair deletion (delTAAT) near the 3 splice-site of exon 3.

Further analyses have indicated that the base exchange c.1321+2T >G results in abnormal splicing and skipping of exon 10. Notably, the c.586+9_12delTAAT deletion was also identified in 2 of 476 unaffected control blood donors, and no aberrant splicing pattern was found in these individuals, strongly suggesting that this deletion is an irrelevant rare variation rather than a pathogenic mutation.

These observations highlight the need for caution in the interpretation of likely splice-site mutations, especially when these variations occur outside the highly conserved donor and acceptor consensus dinucleotides.

P103 FUNTIONAL CHARACTERISATION OF A NEW AMYOTROPHIC LATERAL SCLEROSIS (ALS) ASSOCIATED MUTATION IN VESICLE-ASSOCIATED-MEMBRANE-PROTEIN-ASSOCIATED PROTEIN B (VAPB)

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Keywords: familial amyotrophic lateral sclerosis, VAPB, gene mutations

Background: ALS is a complex degenerative disease selectively affecting motor neurones. A point mutation (P56S) in VAPB has been identified in a large Brazilian pedigree which causes a wide spectrum of motor neurone degenerative disease phenotypes including atypical and typical ALS (1). No other VAPB mutations associated with disease have been identified in other ALS populations (2, 3).

Objectives: To characterise a potential new disease-associated mutation found in the UK and to investigate its pathogenic mechanisms.

Methods: In the first part of this study, 107 non-SOD1 familial ALS (FALS) families were screened for mutations in known ALS-linked genes including VAPB. A point mutation causing an amino acid change from threonine to isoleucine at codon 46 (T46I) was identified in one FALS family but not in 119 controls. To evaluate the effect of this mutation, N-terminal GFP fusion constructs (VAPB, T46I VAPB and P56S VAPB) were generated and expressed in COS-7 and neuroblastoma (N2a) cell lines.

Results: The expression of GFP fusion VAPBs in COS-7 cells showed that the T46I point mutation caused an effect on VAPB protein distribution that was distinct from wild type VAPB and P56S mutant VAPB. Unlike the distribution of wild type VAPB which showed an ER-like localisation, both P56S and T46I showed different degrees of aggregation ranging from small granules to large coalesced structure, the later being more prominent in P56S. In N2a cells, a similar pattern was found, but in a proportion of cells the mutant protein retained an ER-like localization in combination with granules. In order to examine whether the abnormal VAPB distribution affected cell viability, we quantified cell death

using flow cytometry. After correcting for transfection efficiency, there was no difference in cell death between cells expressing wild type or new paramutant VAPB.

Discussion and Conclusion: This is the first report of an ALS-associated mutation in VAPB found in a non-Brazilian population. Like P56S, T46I is located in a very conserved region in the N-terminal MSP domain. The distribution study showed that T46I caused a mild but distinct effect on VAPB protein localisation. Whether this effect will affect protein function is under investigation. Recently, evidence of ER stress in ALS patients and animal models has been reported (4) and VAPB was shown to interact with the ER stress transducer, ATF6 (5). We are currently investigating the effect of T46I on the unfolded protein response pathway which is affected by P56S (6).

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P104 FUNCTIONAL CHARACTERISATION OF PKD1L2, A CANDIDATE GENE IMPLICATED IN MUSCLE DENERVATION AND AXONAL REGENERATION

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Keywords: mouse, genetics, denervation

Background: We have exploited *N-ethyl N-nitrosourea* (ENU) mutagenesis to identify new mouse models and thus novel genes involved in motor neuron loss and muscular atrophy. *Ostes* is an ENU-induced genetic mouse mutant which displays complex neuromuscular phenotypes including denervation, polyinnervation, muscular atrophy and delayed axonal regeneration.

Objectives: We aim to identify the genetic defect and characterise the underlying neuromuscular pathology in *ostes* mice. This would further understanding of the genetic and physiological causes of MND and neuromuscular disorders, and could suggest new genetic pathways for therapeutic intervention.

Methods: cDNA mutation screening was carried out by amplification and sequencing of cDNA from wildtype and *ostes*/*ostes* mice. PKD1L2 $^{-/-}$ mice were generated by injection of a PKD1L2 gene-trapped ES cell line into blastocysts. Protein analysis was carried out by immunoprecipitation, Western blot, mass spectrometry and immunofluorescence. Microarray analysis was carried out on mRNA from wildtype and *ostes*/*ostes* skeletal muscle.

Results: Previously we presented our findings on the genetic mapping of *ostes* and the functional characterisation of a candidate gene, the polycystic kidney disease-1-like gene PKD1L2 (18th Symposium on ALS/MND, Toronto 2007) including protein analysis and transgenic work. Following a round of genomic sequencing including all exons in the non-recombinant region which failed to identify any mutation, we are now undertaking a major cDNA sequencing effort to analyse the cDNA of the full complement of genes in the *ostes* non-recombinant region for mutations that affect mRNA splicing. To further assess the candidacy and function of PKD1L2, we have generated PKD1L2$^{-/-}$ knockout mice using a gene-trapped ES cell line to assess if PKD1L2 deficiency recapitulates the *ostes* phenotype. Furthermore, we have also generated PKD1L2^{-}/ *ostes* double heterozygotic mice to test for non-complementation. Lastly, we have further investigated the function of PKD1L2 and have identified fatty acid synthase (FASN) as an interacting partner of PKD1L2. Interaction has been confirmed by reciprocal co-immunoprecipitation and co-localization experiments. These experiments suggest a role for PKD1L2 in the regulation of fatty acid or lipid metabolism. Consistent with these findings, expression profiling of muscles from *ostes*/*ostes* mice demonstrates significant expression changes of genes involved in lipid metabolism.

Conclusion: Our results indicate that defective or misregulated PKD1L2 causes complex neuromuscular defects in mice. We suggest that PKD1l2 is essential for the regulation of muscle innervation and function and is therefore a candidate gene for uncharacterised forms of motor neuron disease. The direct interaction between FASN and PKD1L2 and the secondary increase of FASN protein observed in *ostes*/*ostes* mice suggest that dysregulation of lipid metabolism may play a role in the pathology of this disease.

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THEME 5 HUMAN CELL BIOLOGY & PATHOLOGY

P105 SKELETAL MUSCLE INVOLVEMENT IN SPINAL AND BULBAR MUSCULAR ATROPHY

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Keywords: spinal and bulbar muscular atrophy, skeletal muscle, neurotrophic factor

Background: Spinal and bulbar muscular atrophy (SBMA) is a hereditary motor neuron disease caused by an expansion of a trinucleotide CAG repeat encoding the polyglutamine tract in the androgen receptor (AR) gene. Testosterone-dependent nuclear accumulation of the pathogenic AR plays a pivotal role in the pathogenesis of SBMA, resulting in the fact that this disease exclusively occurs in males. The pathogenic AR is expressed not only in the nervous system but in non-neuronal tissues, providing the molecular basis of systemic complications such as liver dysfunction and diabetes. Most patients demonstrate a high serum level of creatinine kinase, implying myopathy in SBMA.

Objectives: The aim of this study is to elucidate the skeletal muscle pathology and its clinical implication in SBMA.

Methods: Histopathological analysis was performed on the autopsy specimens of the skeletal muscle from SBMA patients and that from model mice. The intramuscular levels of trophic factors are measured with immunoblotting and immunohistochemistry. The levels of growth factor receptors in lower motor neurons are analyzed with immunohistochemistry of the spinal cord.

Results: Histopathological analysis demonstrated the mixture of neurogenic and myogenic changes in the skeletal muscle. Anti-polyglutamine immunohistochemistry showed abnormal accumulation of the pathogenic AR containing the expanded polyglutamine tract in the skeletal muscles of the SBMA patients and in those of transgenic mice. Protein levels of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), are significantly decreased in the skeletal muscles from the SBMA patients, although the mRNA levels of these factors were not reduced. The expression levels of the receptors for neurotrophic factors were not decreased in the motor neurons within the anterior horn.

Discussion and Conclusions: The present study indicates that the accumulation of the pathogenic AR causes the skeletal muscle pathology in SBMA. The muscle involvement leads to a decrease in the protein levels of neurotrophic factors, that might enhance neurodegeneration in this disease. Our observations are likely to be the theoretical basis for trophic factor supplementation in SBMA patients. Therefore, the skeletal muscles appear to be a primary site of pathology as well as an important target of therapy development.

P106 DECREASED SERUM LEVELS OF PROLYL HYDROXYLASE IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: prolyl hydroxylase, collagen, skin

Background: Recent studies of skin collagen in amyotrophic lateral sclerosis (ALS) have shown several abnormalities including decreased diameter, increased solubility of collagen, alterations of cross-linking of collagen, and decreased type IV collagen. Prolyl hydroxylase (PH) is an enzyme involved in collagen biosynthesis. It catalyses the hydroxylation of prolyl residues in procollagen which is uniquely rich in hydroxyproline. PH activity in number of experimental and clinical states has indicated that this enzyme activity is usually increased in conditions associated with an enhanced rate of collagen formation. The changes in serum PH levels are thought to reflect changes in this enzyme occurring in tissues. However, there has been no study concerning serum PH in ALS.

Objectives: To study serum levels of PH in ALS.

Methods: For the measurement of collagen contents of skin, two-3mm biopsy specimens of skin overlying the left biceps were obtained from 15 ALS patients and 15 diseased control subjects. Approximately 0.5mg of each sample was hydrolyzed with 6N HCl *in vacuo* for 24h at 115°C after flushing with N_2. An aliquot of the hydrolysates was analyzed for its hydroxyproline content on a liquid chromatograph configured as an amino acid analyzer, using ninhydrin with color development at 135°C. For the measurement of serum PH levels, blood samples were obtained from 28 ALS patients, 20 diseased control subjects and 21 healthy control subjects. Serum PH levels were measured with a sandwich enzyme immunoassay (EIA) kit that used a mouse monoclonal antibody against beta subunit of human PH.

Results: The collagen content per dry weight of skin in ALS patients (0.77 ± 0.28 nmol/mg) was significantly smaller ($p < 0.001$) than in diseased control subjects (1.69 ± 0.20 nmol/mg). The collagen content in ALS showed a progressive decrease in relation to duration of illness. This negative correlation was highly significant ($r = -0.75$, $p < 0.01$). Serum PH levels were significantly smaller ($p < 0.001$ and $p < 0.001$) in ALS patients than in diseased and healthy control subjects. There was a significant negative correlation ($r = -0.91$, $p < 0.001$) between serum PH levels and duration of illness in ALS patients. In addition, there was a significant positive correlation ($r = 0.61$, $p < 0.02$) between serum PH concentrations and the collagen content of skin in ALS patients.

Discussion and Conclusions: The present study showed that serum PH concentrations were markedly decreased in ALS patients, which became more pronounced in proportion to the duration of illness. These results indicate a shift in the balance of collagen metabolism towards the direction of a low rate of collagen synthesis in ALS patients. These data suggest

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that decreased serum PH levels reflect decreased collagen content of skin in ALS patients, and that serum PH may be useful in examining the relationship between the formation and the degeneration in skin in ALS.

P107 CIRCULATING ENDOTOXIN AND SYSTEMIC IMMUNE ACTIVATION IN SPORADIC AMYOTROPHIC LATERAL SCLEROSIS (SALS)

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Keywords: Amyotrophic Lateral Sclerosis (ALS); Monocyte/macrophage activation; Lipopolysaccharide (LPS)

Background: Recent studies on blood specimens from patients with sALS found elevated levels of abnormally activated monocyte/macrophages (MO). Lipopolysaccharide (LPS) is a systemic macrophage activator that when injected into animals leads to acute neuron cell death and chronic neuroinflammation/neurodegeneration. More specific to ALS, the injection of LPS into SOD1G37R ALS mice model caused a dramatic shortening of their lifespan suggesting that LPS mediated macrophage activation may exacerbate the pathogenesis of ALS *in vivo.* In an attempt to identity why MOs were activated in ALS patient blood, we evaluated levels of circulating endotoxin (LPS) in the plasma of patients with sALS and compared those results with degree of MO activation and stage of ALS disease.

Objectives: 1) To quantify levels of plasma LPS in sALS patients as compared to control groups, 2) To test whether levels of plasma LPS in blood would correlate with MO activation and/or MO IL-10 expression, and 3) To determine if LPS levels in plasma correlated with clinical stage of disease in sALS.

Methods: Flow cytometry and LAL assay were performed to quantify blood monocyte markers and plasma LPS levels in heparinized blood samples from 13 sALS patients, 13 Alzheimer's (AD) patients, and 13 normal controls (NC). Results from immune studies were evaluated in light of the severity of neurological impairment as determined by ALSFRS-R scores.

Results: Compared to normal plasma (21 ± 7 pg/ml), significantly higher levels of plasma LPS were identified in sALS (42 ± 19 pg/ml, $p < 0.05$) and AD (62 ± 41 pg/ml, $p < 0.01$) patients. Plasma LPS levels between two disease groups of sALS and AD were similar. Higher levels of MO activation were observed in sALS (Mean CD14DR $= 738 \pm 242$, $p < 0.05$) and AD (Mean CD14DR $= 1018 \pm 445$, $p < 0.001$) as compared to NC (Mean CD14DR $= 501 \pm 113$). Analysis of MO activation markers showed that MO HLA-DR expression varied in a direct relationship to plasma LPS levels in disease groups (Pearson $r = 0.7093$, $p < 0.0001$). There was a significant negative relationship between plasma LPS and levels of MO IL-10 expression in sALS blood (Pearson $r = -0.8861$, $p = 0.0001$), but not in the AD and NC groups. Levels of plasma LPS were significantly higher in patients with severe impairment (ALSFRS-R score = 25–36, $n = 6$; LPS $= 52 \pm 17$ pg/ml) compared to NC ($p < 0.01$) and patients with milder impairment (ALSFRS-R score = 37–48, $n = 6$; LPS $= 34 \pm 20$ pg/ml, $p < 0.05$); but no difference was found between NC and patients with milder impairment.

Conclusions: This study, for the first time, reveals that circulating endotoxin levels in patients with sALS may be associated with ALS disease progression. The coupling of elevated LPS levels with loss of MO IL-10 expression suggests that endogenous anti-inflammatory capabilities are defective in patients with sALS. Systemic LPS levels and LPS activated MOs represent two new co-factors that may play significant roles in the pathogenesis of ALS and as such represent novel targets for therapeutic intervention in patients with ALS.

P108 EVIDENCE OF ANTIOXIDANT DEFENSE IMPAIRMENT IN LYMPHOCYTES FROM SPORADIC ALS PATIENTS

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Keywords: reactive oxygen species, lymphocytes, Bcl-2/SOD1

Background: Supposed pathogenic mechanisms involved in amyotrophic lateral sclerosis (ALS) have been recently summarized in two main hypotheses. The former proposes that mutated Cu, Zn superoxide dismutase (SOD1) produces increased oxidative stress; the latter suggests that modified SOD1 would undergo aggregation with other proteins (1), such as bcl-2 (2), thus acquiring a toxic activity. Both hypotheses have recently been supported in sporadic cases (3,4), suggesting a common pathogenic mechanism for both familial and sporadic ALS. In previous work, we provided evidence of a functional impairment of mitochondria, calcium metabolism and SOD1 and bcl-2 expression in lymphocytes from sporadic ALS (SALS) patients, indicating that peripheral tissues may provide information about ALS pathogenesis (5,6).

Objectives: The aim of this work was to evaluate the amount and the time course of oxidative stress, bcl-2 and SOD1 expression levels in lymphocytes of SALS patients.

Methods: SOD1 and bcl-2 expression, reactive oxygen species (ROS), cell damage and apoptosis were evaluated in lymphocytes isolated by Ficoll gradient from peripheral blood of 15 SALS patients and healthy controls. Western blotting with protein extracts using anti-SOD1 and anti-bcl-2 antibodies was performed. ROS, cell damage and apoptosis were determined by flow cytometry using the fluorescent dyes dihydrorhodamine 123, Hoescht33342/Iodium propide and Annexin V.

Results: At the diagnosis, SOD1 and Bcl-2 expression was significantly lower in patient (SOD1: 1.29 ± 0.12, arbitrary units (A.U.); bcl-2: 1.61 ± 0.29, A.U.) than in control (SOD1: 2.28 ± 0.22, A.U., $p < 0.05$; bcl-2 3.83 ± 0.53, A.U., $p < 0.05$) lymphocytes, and decreased during disease progression in a statistically significant way ($p < 0.05$). Flow cytometry technique demonstrated that SALS lymphocytes had significantly higher ROS level and were more damaged and "apoptotic" compared to control cells ($p < 0.05$). The same analyses

performed after about 12 months from diagnosis showed no significant differences for all three assays.

Discussion: The evidence of a progressively reduced SOD1 and bcl-2 expression suggests that cellular pathways involved in the onset of both familial and sporadic forms may be coincident, as hypothesized by others (3,4). Moreover, a deregulation of the antioxidant pathway and abnormalities typical of ALS are present also in lymphocytes, as already suggested by us (6).

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P109 FUNCTIONAL CHARACTERIZATION OF TDP-43 MUTATIONS IN SPORADIC AND FAMILIAL ALS PATIENTS

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Keywords: TDP-43, Aggregation, Animal models

TDP-43 was identified as a key component of ubiquitinated aggregates in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Pathological examinations of autopsy tissue have established TDP-43 labeling in most of the neuronal and glial inclusion bodies observed in these two disorders; and in some other neurological disorders. The *TARDPB* gene (accession number NM_007375) which encodes TDP-43 has been screened in a cohort of ALS patients originating from either France or Quebec to establish whether genetic variants of this gene existed and to investigate whether these could explain the presence of TDP-43 in pathological aggregates. The coding regions of all five exons and of ~50 bp of each of the flanking introns of *TARDBP* were sequenced in each ALS patient. We recently reported eight missense mutations in nine individuals — six cases of sporadic ALS (SALS) and three cases from individuals with a family history of ALS (FALS). Bioinformatics software developed to evaluate the impact of amino acid changes predicts that the mutations are likely to affect post-translational modifications and aggregation propensity of the protein. Also using lymphoblasts cell lines derived from ALS patients with TDP-43 mutations, we observed a significant increase of a smaller TDP-43 product (~25 kDa) alongside the normal product (43 kDa). We further noted that this smaller TDP-43 product was not only specific to individuals were it was mutated (when compared to wild-type TDP-43), but that it was only observed in the detergent insoluble fraction of protein lysates and not in the soluble fraction. We are now in the process of establishing cellular and *in vivo* models were mutant TDP-43 will be transiently expressed to *i*- examine if the mutant form of TDP-43 will lead to the formation of cytoplasmic aggregates which will contain the mutant protein; *ii*- to test if the role of TDP-43 in splicing is affected by these mutations and *iii*- investigate if the protein normal interactions are altered. Altogether these experiments will help to establish whether these mutations lead to an abnormal gain function of mutant TDP-43 or whether they lead to a loss of function of the protein.

P110 EXTRANEURONAL TDP-43 PROTEIN AGGREGATES IN INCLUSION BODY MYOSITIS

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Keywords: TDP-43, Inclusion body myositis, autophagy

Background: The pathogenetic role of TDP-43 in neurodegeneration was first suggested from its presence in ubiquitinated inclusions in sporadic and non-SOD1 familial ALS. A genetic aetiology was attributed to ten missense mutations of TDP-43 in ALS. TDP-43 is highly conserved and ubiquitously expressed, but its prevalence in muscle pathology is not known. Inclusion body myositis (IBM) is the most common debilitating muscle disease over the age of 50. Its pathogenesis is unclear; a major issue in IBM pathology is its degenerative nature. The presence of abnormal protein aggregates like beta amyloid and tau protein in IBM strongly supports a degenerative paradigm, but the invasion of cytotoxic T cells and upregulation of MHC I antigen suggest an inflammatory component.

Objectives: To explore the extraneuronal presence of TDP-43 pathology and its possible role in the pathogenesis of IBM.

Methods: Muscle biopsy tissues from pathologically confirmed IBM cases were used for immunohistochemistry (IHC), confocal and Western blot analysis. Normal human muscle biopsies were used as controls. Genomic DNA was extracted from muscle samples, amplified with Taq Gold DNA polymerase, then sequenced on the Beckman Coulter CEQ 8000 Series Genetic Analysis System.

Results: Ten IBM cases were studied. TDP-43 aggregates were present in the IBM rimmed vacuoles. IHC of serial muscle sections showed localization of TDP-43 in the same vacuoles with other proteins including beta-amyloid, Tau, valosin-containing protein (VCP), oligo- and multi-ubiquitin, microtubule-associated protein light chain 3 (LC3B) and p62. The colocalization was further validated by confocal microscopic examinations. The sequencing of TDP-43 and western blot analysis are currently underway.

Discussion and Conclusions: This is the first report of extraneuronal presence of TDP-43 pathology and might be of pathological and clinical relevance not only to TDP-43 proteinopathy and also to muscle pathology. The finding of colocalization of TDP-43 with beta-amyloid and tau lends more weight to the degenerative nature of IBM. VCP is causative of hereditary inclusion body myopathy with Paget's disease of the bone and frontotemporal dementia (IBMPFD). The colocalization of TDP-43 and VCP suggests complex pathways in protein regulation in the cell. LC3B, a homologue of yeast Atg8, is an essential component and marker of the autophagy. p62 may link polyubiquitinated protein aggregates to the autophagy machinery. The colocalization of TDP-43 with LC3B and p62 might suggest a role of autophagy in the degradation of TDP-43 and IBM pathogenesis. The undergoing sequencing and Western blot analysis will clarify more on the pathogenic roles of TDP-43 in IBM.

P111 FAMILIAL ALS: CLINICOPATHOLOGICAL CORRELATES

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Keywords: genetics, pathology, clinical

Background: We have now conducted linkage studies using single nucleotide polymorphism (SNP) DNA microarrays in a number of ALS kindreds and have previously reported our identification of TDP-43 mutations in one family. Since then further groups have identified TDP-43 mutations.

TDP-43 inclusions are seen in both ALS and frontotemporal dementia with ubiquitinated inclusions (FTLD-U) and both conditions have significant clinical overlap as well. It is therefore of note that no mutations have thus far been described in FTLD-U kindreds. Interestingly, none of the published kindreds with TDP-43 mutations have features of dementia. TDP-43 mutations still only account for a small number of fALS cases and, although a key discovery, the continued study of other fALS kindreds is imperative.

Objectives: To correlate clinical and pathological features in fALS kindreds.

Methods: fALS cases were identified and examined by consultant neurologists. Clinical and neurophysiological data were also obtained retrospectively from case notes. Pathological studies were conducted using immunohistochemistry (including TDP-43 and ubiquitin staining) and immunoblotting.

Results: Clinical phenotype varied dramatically within one particular kindred in which typical ALS, PLS and ALS-FTD were all seen, although there was insufficient power to detect linkage and post-mortem tissue was not available. Conversely, in two other kindreds, affected individuals had a strikingly similar clinical phenotype. This is in clear contrast to mutant SOD1 fALS where phenotypic heterogeneity is seen (the D90A mutation being an exception). Pathological studies were successfully conducted on brains and spinal cords from selected cases from these two families.

Discussion and Conclusions: ALS can present with bulbar or limb, upper or lower motor neuron features, and it is increasingly clear that there is an overlap with FTD. The families we present demonstrate phenotypic variability and stereotypy. Pathological studies are important and our results demonstrate that correlating post-mortem data with clinical phenotype will provide valuable insight into disease pathogenesis.

P112 THE EXPRESSION OF TAU pThr^{175} IN ALS AND ALS WITH COGNITIVE IMPAIRMENT (ALSCI)

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Keywords: frontotemporal dementia, tau, phosphorylation, ALSci

Background: We have previously demonstrated the neuropathological hallmark of ALS with cognitive impairment (ALSci) is the presence of a frontotemporal lobar degeneration in association with both intracellular (neuronal and astrocytic) and extracellular tau deposition. We have further identified that tau isolated from ALSci is uniquely phosphorylated at pThr^{175} and that tau-aggregate bearing neurons also over-express GSK3β.

Objectives: To determine whether the expression of tau pThr^{175} in ALS and ALSci is dependant upon the CNS region of interest, we have developed a rabbit polyclonal antibody to tau pThr^{175} and compared its immunoreactivity to either traditional tau antibodies or Gallyas staining.

Method: 6 micrometer paraffin-embedded sections of the superior frontal gyrus, anterior cingulate gyrus, amygdala, hippocampus, entorhinal cortex, basal ganglion and substantia nigra were taken from neuropathologically confirmed ALS (5) and ALSci (5) patients. Sections were immunostained by using rabbit polycolonal tau antibody against pThr^{175} without antigen retrieval. Endogenous peroxidase was quenched with 3% hydrogen peroxide (BDH). The primary antibody was incubated at 4°C overnight. Tissue sections were incubated in 1:200 biotinylated anti-rabbit IgG secondary antibody for 1 hour at room temperature followed by substrate development using the Vectastain ABC kit as per the manufacturers instructions. The extent of pThr^{175} immunostaining was described on the basis of both distribution and morphology.

Results: Tau pThr^{175} immunoreactive neurons and neurites were observed in the entorhinal, hippocampal, substantia nigra and amygdale of ALS and ALSci. Either punctate, pretangled or tangled neuronal deposition was observed in the entorhinal cortex and the hippocampus, and as a granular deposition in the substantia nigra. In ALci, tau pThr^{175} immunoreactive inclusions were mainly distributed in the superficial layers of the entorhinal cortex, in regions CA1 and CA2 of the hippocampus, and throughout the substantia nigra. pThr^{175} tau neuronal deposition was occasionally observed in the superior frontal gyrus and the anterior cingulate gyrus of ALSci, but not in ALS. In addition, tau pThr^{175} immunoreactive oligodendroglia were occasionally seen in the deeper cortical layers of the superior frontal gyrus of ALSci. No pThr^{175} immunoreactive neuritic plaques exist in these given regions. Tau pThr175 immunoreactive pathology was rarely observed in ALS.

Conclusions: Using a polyclonal antibody directed to an ALSci-specific tau phospho-epitope (tau pThr^{175}), we have observed tau immunoreactive inclusions in a widely distributed pattern in ALS. Of note, only ALSci demonstrated tau pThr175 immunoreactivity in the superior frontal gyrus and anterior cingulate gyrus of ALS.

Research supported by the Scottish Rite Charitable Foundation.

P113 PSEUDOPHOSPHORYLATION OF TAU AT THREONINE 175 (^{175}THR) IN ALSCI IS ASSOCIATED WITH TAU FIBRIL FORMATION *IN VITRO*

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Keywords: tau, phosphorylation, frontotemporal dementia

Background: Amyotrophic lateral sclerosis with cognitive impairment (ALSci) is associated with many of the neuropathological features of a frontotemporal lobar degeneration, including tau deposits (1). We have shown that tau isolated from ALSci patients differs from neurologically normal (control) or Alzheimer's disease (AD) tau in that it is uniquely phosphorylated at ^{175}Thr (pThr^{175} tau)(2). We have also shown that this is associated with an up-regulation of GSK-3β expression in tau-aggregate containing neurons in ALSci, suggesting that this process may be responsive to pharmacological manipulation (e.g., lithium responsive) (3).

Objectives: We have determined whether tau phosphorylation at ^{175}Thr is associated with tau fibril formation *ex vivo* using tau isolated from ALS, ALSci and Alzheimer's disease (AD) and compared this to tau fibril formation *in vitro*, and whether this impacts on neuronal survival.

Methods: Both soluble and insoluble tau protein was purified from control, ALS, ALSci and AD by HPLC and fibril formation assayed *ex vivo* using the thioflavin S fluorescence assay. Using both Neuro2A and HEK293T cells, we expressed full-length EGFP-tagged tau constructs harbouring either a pseudophosphorylation at ^{175}Thr (Thr^{175}Asp-tau), loss of the phosphorylation site (Thr^{175}Ala-tau) or wild-type tau (*wt*-tau). The extent of tau fibril formation was assayed using confocal microscopy, and correlated with the extent of cell death (assayed using MTT assay and activated caspase 3 immunoreactivity). As well, the association of each with β-tubulin was assayed immunohistochemically using confocal microscopy.

Results: The extent of fibril formation as assessed with the thioflavin S assay was significantly greater in tau derived from ALSci, while tau isolated from ALS showed intermediate fibril formation between that of control and AD-derived tau. We next examined the association between pThr^{175} tau (using EGFP-Thr^{175}Asp/Ala or *wt*-tau) and the formation of intraneuronal fibrils *in vitro*. Both fibril formation (as measured by the presence of EGFP-positive fibrils instead of punctate staining by confocal microscopy) and cell death (as measured by MTT and activated caspase 3 immunocytochemistry) were significantly enhanced in the presence of Thr^{175}Asp-tau, regardless of the tau isoform. We also observed that *wt*-tau colocalizes with β-tubulin, while Thr^{175}Asp-tau fails to colocalize.

Conclusions: These data suggest that the unique phosphorylation at ^{175}Thr in ALSci is associated with increased tau fibril formation, and that phosphorylation at this site can disrupt the normal interaction of tau with β-tubulin.

Research supported by the Scottish Rite Charitable Foundation.

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P114 NOVEL ANTIBODIES REVEAL INCLUSIONS CONTAINING MISFOLDED SOD1 IN ALS PATIENTS

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Keywords: SOD1, sporadic ALS, spinal cord

Background: Mutations in CuZn-superoxide dismutase (SOD1) cause amyotrophic lateral sclerosis (ALS) and are found in 6% of ALS patients. The cause(s) of the disease in the reminder is largely unknown. Misfolded and aggregation-prone forms of mutant SOD1s are thought to trigger the disease. In several other neurodegenerative conditions such as Alzheimer′s, Parkinson′s and Creutzfeldt-Jacob′s diseases, protein that are mutated in some of the familial patients are also involved in the pathogenesis in patients lacking such mutations.

Objective: Could wild-type SOD1, by analogy, be involved in ALS patients lacking SOD1 mutations?

Methods: Two sets of novel antibodies, raised in rabbits and chickens, against peptides spaced along the human SOD1 sequence, were developed and shown to be specific for denatured SOD1. These were used to examine SOD1 in spinal cords of ALS patients lacking mutations in the enzyme.

Results: Small granular SOD1-immunoreactive inclusions were found in spinal motoneurons of all 37 sporadic and familial ALS patients studied, but only sparsely in 3 of 27 neurodegenerative and 2 of 19 non-neurological control patients. The granular inclusions were by confocal microscopy found to partly colocalize with markers for lysosomes but not with inclusions containing TAR DNA binding protein-43, ubiquitin or markers for endoplasmatic reticulum or mitochondria. Granular inclusions were also found in carriers of SOD1 mutations and they were the major type of inclusions detected in ALS patients homozygous for the wild type-like D90A mutation.

Conclusion: The findings suggest that SOD1 may be involved in ALS pathogenesis in patients' lacking mutations in the enzyme.

P115 ROLE OF THE TRANSCRIPTIONAL CO-ACTIVATOR PGC-1α IN ALS?

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Keywords: PGC-1alpha, oxidative stress, mitochondria

Background: Cell death induced by reactive oxygen and nitrogen species (ROS, RNS) has been shown to be a pathomechanism in both familial and sporadic ALS. Mitochondria are the major source of cellular ROS production, which further increases if mitochondria are damaged (1, 2). The transcriptional co-activator peroxisome proliferator-activated receptor-γ co-activator 1α (PGC-1α) regulates mitochondrial metabolism and biogenesis via activation of transcription factors such as nuclear respiratory factor-1 (NRF-1) (3). It also plays a role in maintaining synaptic function (4). Alterations in PGC-1α expression and function have previously been described in models of Huntington's and Alzheimer's disease (5, 6).

Objectives: In the present study, we investigated the mRNA and protein expression of PGC-1α and NRF-1 in human post mortem tissue of ALS patients as well as in the G93A-ALS mouse model in presymptomatic, early –and late symptomatic stages.

Methods: We performed in situ hybridization histochemistry, quantitative real time PCR, immunohistochemistry, and Western blot experiments on human post mortem tissue of ALS patients and age matched controls and on spinal cord tissue from the G93A mouse model. We also studied PGC-1α expression and subcellular localization in primary motor neuron cultures derived from transgenic ALS mice and non-transgenic littermates.

Results: Both in post mortem ALS tissue and in G93A mice, we observed a reduction of PGC-1α and NRF-1 at the mRNA and protein level, in the animal model already detectable before symptom onset.

Discussion and Conclusion: We therefore conclude that a decrease in PGC-1α could contribute to reduced antioxidant defense mechanisms in familial and sporadic ALS and that it may represent an interesting therapeutic target.

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P116 IMMUNOHISTOCHEMICAL EXPRESSION OF IGF-I AND GSK IN THE SPINAL CORD OF KII AND GUAM ALS PATIENTS

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Keywords: IGF, GSK-3α/β; focus ALS

Background: Insulin-like growth factor-I (IGF-I) is a potent survival factor for motor neurons in animals and glycogen synthase kinase-3β (GSK-3β) is suspected to have a role in inflammation, apoptosis and tau phosphorylation. However, little information is available on changes in IGF and GSK signalling pathways in ALS patients.

Objectives: We examined the immunological expression for IGF-I, GSK-3β and phospho (p)-GSK-3α/β in the spinal cord and the hippocampus of Kii and Guam ALS patients.

Methods: Sixteen ALS patients (10 sporadic Japanese ALS patients, 3 Kii ALS patients, 3 Guam ALS patients) and 12 neurological controls (8 Japanese patients, 4 Guam patients) were examined. Paraffin embedded sections 8-μ m thick were stained with the immunohistochemical technique using the ABC system (Vector Laboratories, Inc., Burlingame, CA, USA). The immunoreactivity for each antibody was scored as negative (0), slightly positive (1), mildly positive (2) and marked positive (3). Antibodies used in the present study were mouse anti-IGF-I monoclonal antibody (CEHMICON INTERNATIONAL, USA), anti-GSK-3β monoclonal and rabbit anti-GSK-3α/β polyclonal antibodies (CELL SIGNALLING TECHNOLOGY, USA).

Results: Motor neurons in the spinal cords from the Japanese neurological controls were mildly positive with anti-IGF-I and anti-GSK-3β antibodies and were slightly positive for anti-p-GSK-3α/β antibody. Those from Guam controls also showed mildly positive for anti-IGF-I and anti-p-GSK-3α/β antibodies. Spinal motor neurons from the sporadic Japanese ALS patients were almost negative for anti-IGF-I antibody, but mildly positive for anti-GSK-3β and anti-p-GSK-3α/β antibodies; however, those from Kii ALS patients with a long clinical duration showed marked positive for anti-IGF-I and p-GSK-3α/β antibodies. Spinal motor neurons from Guam ALS patients with short clinical durations were almost negative for anti-IGF-I and anti-GSK-3β, but marked positive for anti-p-GSK-3α/β antibodies. Ratios for immunoreactivity scores of GSK-3β to p-GSK-3α/β in the spinal motor neurons were different between Kii and Guam ALS patients and Japanese sporadic ALS patients ($p < 0.01$). Immunological co-localization of IGF-I and p-GSK-3α/β was seen in spinal motor neurons from Kii ALS patients using confocal laser scanning. Neurofibrillary tangles in the hippocampus from Kii and Guam ALS patients were positive for anti-tau and anti-p-GSK-3α/β antibodies, while negative for anti-GSK-3βantibody.

Discussion and Conclusion: Positive immunoreactivity for IGF-I might be related to the clinical duration of ALS. Kii and Guam ALS patients showed a similar immuno-staining pattern for GSK-3β and p-GSK-3α/β which was different from the Japanese sporadic ALS patients. The predominant expression of p-GSK-3α/β compared to GSK-3β in the spinal motor neurons and NFT-laden neurons in the hippocampus was a characteristic finding in Kii and Guam ALS patients. The role of IGF signal pathway and GSK-3βphosphorylation in the focus ALS patients should be pursued.

P117 IN SITU DETECTION OF DPP6 EXPRESSION IN THE CENTRAL NERVOUS SYSTEM WITH A NOVEL EFFICIENT IN SITU HYBRIDISATION TECHNIQUE USING 2OME AND LNA MODIFIED DETECTION PROBES.

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Keywords: gene expression, in situ hybridization, DPP6

Background: LNA (Locked Nucleic Acid) modified DNA oligonucleotides are used to detect miRNA in situ hybridisations. Short oligonucleotides which are fully modified with alternating LNA and 2'-O-Me-RNA (2OME) RNA moieties have very high affinity for matching RNA strands much stronger than the corresponding LNA-modified DNA oligos. We hypothesized that the strong and specific RNA binding of these 2OME/LNA modified oligos would make them ideal for microRNA (miRNA) and mRNA in situ detection. We have used these oligos to develop a "one day" in situ hybridisation protocol allowing detection of both miRNA and mRNA expression in paraffin embedded archival material.

Objectives: To setup a simple hybridisation protocol which can be performed in one day to detect either miRNA or mRNA in archival paraffin embedded samples using the new LNA 2OME modified short oligonucleotides as probes.

Methods: The probes are constructed as 19mer LNA-modified 2OME-RNA oligos with every third base as LNA and the remaining nucleotides as 2OME-RNA. The combination of LNA with 2OME RNA raises the Tm of the oligo with nearly 1.5°C per LNA modification as compared with LNA-DNA oligonucleotides. The oligo sequences were designed to be specific for the target sequences. The LNA/2OME oligo's are 5′ labelled with FAM. The FAM label allows detection and amplification of the signal using a secondary antibody against FAM. For this study we designed LNA/2OME RNA oligo's against a miRNA (mir-134) and a mRNA (DPP6). We tested the specificity of these LNA/2OME RNA oligo's in archival paraffin embedded brain and spinal cord samples. The high affinity of the LNA/2OME probes allows for one hour hybridisations, meaning that the whole protocol can be easily done within one day.

Results: Both the mir-134 probe and the DPP6 probe showed strong and specific staining patterns in brain and spinal cord tissue using the short hybridisation protocols.

Both paraffin and frozen sections can be used for this protocol. The signals could be competed for by unlabeled competitor probe indicating specificity. The Mir-134 expression could be detected in pyramidal neurons in the cortex and we could detect high DPP6 levels in neurons in spinal cord and hippocampus.

Discussion and Conclusions: We have set up a convenient and fast in situ hybridisation protocol using new LNA/2OME modified RNA oligo nucleotides. This protocol can be used to detect miRNA and mRNA expression in archival paraffin material.

P118 MICROARRAY ANALYSIS IDENTIFIES THE GENE SIGNATURE OF SPARED VERSUS VULNERABLE MOTOR NEURONE GROUPS UNDERLYING SELECTIVE VULNERABILITY IN MOTOR NEURONE DISEASE (MND)

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Keywords: Selective vulnerability, gene expression, Laser capture

Background: Amyotrophic lateral sclerosis is characterised by the progressive degeneration of upper and lower motor neurones in the motor cortex, brain stem and spinal cord. It leads to skeletal muscle atrophy and weakness, before eventually causing death. The motor neurones affected are primarily alpha motor neurones in the spinal cord and brainstem innervating limb and bulbar muscles, and neurones forming descending pathways of the corticospinal tract. However, other motor neurone groups such as those in the oculomotor nucleus, which innervate the extraocular muscles and Onuf's nucleus in the sacral spinal cord, are spared in the disease (1).

Objectives: This study aims to investigate the differential gene expression profile of spared and vulnerable motor neurone groups, to give insight into the molecular basis of selective vulnerability of motor neurones in the disease process associated with MND.

Methodology: Using human post-mortem tissue from neurologically normal control cases, motor neurones from the lumbar and cervical spinal cord and from the oculomotor nucleus in the brainstem were extracted using laser capture micro-dissection. Total RNA was isolated and amplified using the two step amplification method. Biotin labelled antisense RNA was applied to Affymetrix U133 plus 2 GeneChips and hybridisation, washing and staining were carried out as described previously (2).

Results: The gene expression profiles generated have been compared using the PUMA software programme and differentially expressed genes identified (3). Thus some 102 genes were upregulated and 185 downregulated in the oculomotor nucleus motor neurones compared to spinal motor neurones. These have been further characterised according to gene ontology terms. Hence genes involved in calcium binding, neurotransmitter receptors, transcription factors and cytoskeletal proteins are differentially regulated in the oculomotor nucleus.

Conclusions: The results indicate that the oculomotor motor neurones are indeed different to spinal cord motor neurones and that these differences may account for their reduced vulnerability to death during ALS.

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THEME 6 IMPROVING DIAGNOSIS, PROGNOSIS AND DISEASE PROGRESSION

P119 VALIDATION STUDY FOR PROTEIN BIOMARKERS IN THE CSF OF ALS PATIENTS

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Background: We previously reported the identification of protein biomarkers in the cerebrospinal fluid (CSF) of ALS patients using mass spectrometry based proteomics. Surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS) was performed on CSF collected from 52 control and ALS subjects to identify protein peaks with diagnostic predictive value, with testing on a separate coded set of CSF samples. We further validated two of the biomarker proteins, cystatin C and transthyretin, using immunoblot and immunohistochemistry on separate groups of ALS and control subjects.

Objectives: We performed the current study to validate our initial findings using a much larger set of CSF samples.

Methods: During the past two years we collected 263 additional CSF samples from ALS and control subjects at the University of Pittsburgh School of Medicine and Massachusetts General Hospital. The subjects included ALS, Alzheimer's disease (AD), healthy control, multiple sclerosis, and other neurological diseases including FTD patients. We performed SELDI-TOF-MS on each CSF sample using two Protein chip surfaces, IMAC and Q10. Samples were run in duplicate on separate protein chips and experiments repeated three times. Mass spectra were collected from the 2,000–200,000 Dalton mass range.

Results: We compared the protein profiles from ALS patients to each of the other subject groups and all other subjects. We identified a total of 60 mass peaks with statistically significant alterations between ALS and control subjects ($p < 0.001$). Similar to our prior study, cystatin C and transthyretin protein peaks were decreased in ALS when compared to control subjects. We identified a panel of proteins that predicted ALS versus control subjects with 84% overall accuracy. The putative identification for many of these protein peaks has been determined and includes proteins in protein transport and neuroprotective signalling pathways.

Conclusions: Our data validates prior results indicating decreased levels of cystatin C and transthyretin in the CSF of ALS patients. Additional protein peaks were used to create a predictive biomarker panel that identified ALS with 84% overall accuracy. We further identified specific protein mass peaks that differentiate ALS from AD subjects.

P120 PLASMA BASED PROTEIN BIOMARKERS FOR ALS

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Keywords: plasma, biomarkers, mass spectrometry proteomics

Background: Diagnostic biomarkers for ALS would greatly benefit the rapid diagnosis of the disease, aid in determining the underlying pathogenesis of ALS, and may assist in future clinical trials. Biomarkers discovery efforts typically use biofluids such as blood or cerebrospinal fluid (CSF). We previously reported the identification of protein biomarkers in the CSF of ALS patients using mass surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS) based proteomics. Specific protein biomarkers were obtained that differentiated ALS from control subjects. We next wished to determine if plasma based biomarkers could be identified from the same patient populations. We obtained blood plasma samples from 62 ALS and control subjects and performed SELDI-TOF-MS to identify protein peaks with diagnostic predictive value.

Objectives: To identify protein based biomarkers in the plasma of ALS patients near the time of symptom onset, and compare these biomarkers to those found in the CSF.

Methods: We collected 62 plasma samples from 31 ALS and 31 healthy control subjects at the Massachusetts General Hospital. We performed SELDI-TOF-MS on each CSF sample using two Protein chip surfaces, IMAC and Q10. Samples were run in duplicate on separate protein chips and experiments repeated three times. Mass spectra were collected from the 2,000–200,000 Dalton mass range.

Results: We compared the protein profiles from ALS patients to the control subjects. We identified a total of 14 mass peaks with statistically significant alterations between ALS and control subjects ($p < 0.05$). These proteins ranged in mass from 4.4 to 88.8 kDa. Similar to our prior CSF study, transthyretin protein peaks were altered in ALS compared to control subjects. We identified a panel of 4 protein mass peaks that predicted ALS versus control subjects with 82% overall accuracy using a classification tree analysis. We also used a machine learning algorithm and determined that the 4.4 kDa peak ($p = 0.0004$) provided a 90% positive predictive value for ALS. The 4.4 kDa protein peak was increased in ALS patients relative to control subjects.

Conclusions: We have identified specific protein peaks in the plasma that differentiate ALS from healthy control subjects. Further studies are required to validate these findings and expand them to include additional ALS and other disease control groups.

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P121 QUANTITATIVE SIT-TO-STAND (STSV), 360 DEGREE AXIAL ROTATION (AXRV) AND WALKING (WLKV) VELOCITY IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)-PROSPECTIVE NATURAL HISTORY OF THE COMPONENTS OF THE TIMED UP AND GO TEST INCLUDING CORRELATION WITH FALLS, ADAPTIVE EQUIPMENT NEEDS AND SURVIVAL

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Keywords: Outcome Measure, Rehabilitation, Postural Reflexes

Objective: Perform a factor analysis of the independent constitutive components of the Timed Up-and-Go test, which is proposed as an outcome measure for clinical trials in ALS.

Background: Quantitative Sit to Stand (STSv), 360 Degree Axial Rotation (AXRv) and Walking Velocity (WLKV) were prospectively determined in 227 ALS (129 M; 98 F) at each clinic visit over 9–108 months follow-up.

Methods: STSv is determined by measuring the time (seconds) from the command "stand" to the subject achieving a totally erect stance and dividing the distance from the chair seat to the upright position (2 feet) by this time to give the STSv (Ft/sec). AXRv is determined by measuring the time (seconds) from the command "turn" to completion of a full 360 degree axial rotation clockwise or counter-clockwise resuming the starting stance with the feet in the same starting position. Angular velocity represented as degrees/sec is transformed to Ft/sec by assuming the stance is represented by a radius of 0.5 foot yielding a circumference of 2π-r (3.1 Ft). AXRv is provided in comparable units by dividing the circumference distance travelled by the time to complete the axial rotation. WLKv is determined by measuring the time from "start" to the completion of a 30 Ft walk performed as quickly as the patient safely can. STSv, AKRv, WLKv were analyzed by descriptive statistics cross-sectionally and longitudinally. Changes in STSv, AXRv and WLKv were correlated with sex, age, site of disease onset, spasticity, milestones including more than 2 falls monthly, use of adaptive equipment (cane, walker, wheelchair) and survival (MediCalc).

Results: In subjects with no neurological diagnosis, median 50% STSv is 2.4 Ft/sec with 25–75th quartile range: 1.8–3.2 Ft/sec, median AXRv is 1.8 Ft/sec with 25–75th quartile range: 1.4–2.2 Ft/sec and median WLKv is 7.0 Ft/sec with 25–75th quartile range: 5.4–8.4 Ft/sec. STSv is decreased to a greater degree than AXRv and WLKv in early ALS. AXRv in ALS patients requiring a cane is decreased to greater extent than STSv and especially WLKv. STSv and AXRv together are much more decreased than WLKv in ALS patients requiring a walker. Wheel chair dependent ALS patients have a significantly decreased and usually non-measurable STSv, AXRV and WLKv. Five Year survival is 36% in ALS patients. The proportion of ALS patients with STSv, AXRv and WLKv greater than 50% predicted is comparable at two years.

Conclusions: Timed Up-and-Go Test may be studied through its components. Transformation to STSv, AXRv and WLKv indicates that Sit-To-Stand and Axial Rotation are more severely affected in ALS patients than Walking. STSv and AXRv are more severely decreased than WLKv with development of dependency on cane or walker.

P122 THE DIFFERENT MECHANISMS OF DYSPHAGIA IN BULBAR AND PSEUDOBULBAR PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Keywords: dysphagia, mechanisms, manometry

Background: Swallowing mechanism in ALS patients has not been systematically studied. The oesophageal manometry (OM) can be an objective, manometric method for oesophageal peristalsis failure detection in ALS patients. There is some evidence that lesions of the pseudobulbar tract are more responsible for dysphagia due to ALS.

Objective: We investigated the mechanisms of oesophageal dysphagia in ALS patients with mainly bulbar (BP) or pseudobulbar clinical presentation (PBP).

Material and Methods: 28 (BP = 13 and PBP = 15) sporadic ALS patients with clinical evidence of dysphagia, fulfilling WFN criteria, from Krakow MND Centre, were involved in the study.

The standard transnasal OM was performed in all subjects studied and 20 healthy volunteers (control group) using a flexible catheter with the solid state transducers. The parameters were measured automatically and visualised by the computer system. Swallows were initiated with 5 to 10 ml of water (wet swallows) and saliva (dry swallows) and repeated at 30 second (sec) intervals in all subjects. We compared the upper oesophageal contractile amplitude and duration, rest pressure of the upper oesophageal sphincter, negative pressure of the oseophageal body, propagation of peristaltic waves, the upper oesophageal contractile velocity in ALS patients with BP or PBP and also control subjects.

Results: ALS patients studied had significant abnormalities in all parameters measured by OM as compared to controls. In BP cases we noticed: the upper oesophageal contractile amplitude-max. 38.6 ± 6 mmHg (mean:17 ± 5), the mean upper oesophageal contractile duration-1.4 ± 0.2 sec., rest pressure of the upper oesophageal sphincter-12 ± 2.4 mmHg, the occurrence of the negative pressure of oseophageal body-all cases studied, propagation of peristaltic waves-8 out of 13, the upper oesophageal contractile velocity-2 cm/sec. In PBP cases we detected: the upper oesophageal contractile amplitude-max: 98.6 ± 19 mmHg (mean: 67 ± 15), the mean upper oesophageal contractile duration-5.2 ± 1.5 sec., rest pressure of the upper oesophageal sphincter 55 ± 17 mmHg, the occurrence of the negative pressure of oseophageal body-3 out of 15, propagation of peristaltic waves-3 out of 15, the upper oesophageal contractile velocity-4.3 cm/sec.

Summary: OM procedure allows us to objectively distinguishing dysphagia in ALS patients due to mainly bulbar or pseudobulbar syndrome which could be important for revealing the cases with high risk of aspiration. Our results are in line with data suggesting that dysphagia in ALS cases is mainly associated with progressive degeneration of the corticobulbar pyramidal fibers.

P123 A CLINICAL BULBAR SCALE FOR ASSESSMENT MONITORING OF CHANGE IN ALS

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Keywords: bulbar, scale, clinical, speech

Background: Persons with ALS (PALS) experience rapid progression of spastic-flaccid dysarthria. A clinical bulbar scale would provide a means of identifying UMN and LMN features observed in speech, and evaluate swallowing status. The scale would include timed verbal capacity, sentence intelligibility, communication effectiveness, UMN and LMN features, and swallowing. Timed verbal capacity has been identified as deficient among a subset of PALS. Sentence intelligibility is used to objectively measure speaking performance. Speaking rate is an effective indicator of pending reduction in intelligibility; however, the above evidence does not provide a timeline for predicting speech loss. PALS report that communication effectiveness is functionally impaired when sentence intelligibility is below 90%. When intelligibility declines to 80%, verbal communication is completely ineffective. These features, combined with clinical speech signs, comprise a proposed bulbar scale.

Objectives: A clinical scale for identifying bulbar dysfunction associated with ALS is proposed for use in identification and longitudinal monitoring. Initial face validity and reliability analyses will be completed.

Method: A Kaplan-Meier estimator was used to calculate survival from longitudinal speech intelligibility and rate scores, or PALS' "*speech lifetime*". Data were obtained from a clinical ALS database of 173 PALS over 380 sessions. Participant characteristics included: 94 male:79 female; 52 bulbar:121 spinal onset; mean = 61 years, range 38–72. Speech characteristics were obtained for analysis (e.g., examination date, percent sentence intelligibility, speaking rate). Kaplan-Meier estimates were calculated to evaluate survival of functional speech based on rate and sentence intelligibility. The survival estimate of speech was determined for 50% intelligibility, the level at which participants indicate speech is no longer functional. A second calculation of survival at 80% was based on communication effectiveness ratings obtained from PALS who indicated this level as when speech becomes ineffective.

Results: Data identified speaking rate as an early predictor of bulbar dysfunction. PALS show a decline in intelligibility preceded by a speaking rate decline. Months post ALS diagnosis did not prove predictive of speech loss in either bulbar or spinal ALS. Results indicate 50% intelligibility survival function for all participants (mean = 578.88 days, median = 590) was approximately 1.5 years. The 80% intelligibility survival for bulbar ALS (M = 245.91 days, median = 241) was shorter than that for spinal ALS (M = 522.92 days, Mdn = 301). Data will illustrate survival functions for speaking rate.

Discussion: Intelligibility alone is not an adequate identifier of bulbar speech symptoms in ALS. This study provides the first speech survival times of PALS. Spinal onset PALS have longer speech survival than bulbar; but more importantly, dysarthria onset is preceded by changes in speaking rate and communication effectiveness that are imperceptible during clinical interactions. Validity and reliability of the bulbar scale will be presented, with research and implications discussed.

P124 MEASURES OF MAXIMUM PERFORMANCE OF SPEECH-RELATED TASKS IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: Duration, rate, dysarthria

Background: Measures of maximum performance of speech-related tasks are often used to determine the efficiency of the oral –vocal motor system.

Objectives: To evaluate measures of maximum performance of speech-related tasks in Amyotrophic lateral sclerosis (ALS).

Methods: Participants of the study included 70 patients with ALS (46 spinal onset and 24 bulbar onset) and 58 healthy volunteers (controls). The patients and controls were compatible with reference to age (25–73 yrs), gender and four Indian vernacular languages (native speakers of Kannada, Tamil, Telugu; and Hindi speakers of Indo-Aryan language background). All participants were tested on each of the following tasks i.e. Maximum Phonation Duration (MPD) of (a); Maximum Frication Duration (MFD) of (s) and (z); Diadochokinetic (DDK) rate of (pa), (ta), (ka) and (pataka); and articulatory precision of (pa), (ta) and (ka) during the task of DDK rate.

Discussion and Conclusion: Mean age (years) of patients was 49.47 ± 10.56 and controls was 47.48 ± 11.08. M: F- 3.4:1. Spinal onset: bulbar onset ALS- 2.3:1. In spinal onset group, mean durations of speech symptoms and limb symptoms were 3.89 ± 5.05 and 17.26 ± 12.61 months respectively. In bulbar onset group, mean durations of speech symptoms and limb symptoms were 13.90 ± 12.12 and 4.13 ± 5.83 months respectively. Overall mean duration of illness was not significantly different among patients speaking the four vernacular languages.

Mean MPD (seconds) of (a) for patients was 11.87 ± 6.1 while in controls, it was 19.33 ± 6.28. Mean MFD (seconds) for (s) was 5.53 ± 5.44 in patients and 11.76 ± 4.91 in controls. Mean MFD for (z) was 6.34 ± 6.61 seconds in patients while in controls, it was 14.27 ± 4.41. Mean DDK rate (syllables per second) of (pa), (ta), (ka) and (pataka) in patients was 4.25 ± 1.8, 4.17 ± 1.94, 3.98 ± 1.92 and 6.04 ± 2.9 respectively; and in controls was 6.69 ± 0.77, 6.96 ± 0.84, 6.59 ± 0.74 and 8.94 ± 1.35 respectively.

A significant difference ($p < 0.05$) in the performance was observed for all parameters between control group and the patient group; control group and the spinal onset group; control group and bulbar onset group. A significant difference between spinal onset and bulbar onset groups was also evident on all the parameters except MPD. There was no significant difference in the DDK rates of (pa), (ta), (ka) and (pataka) in the patient group across the four different languages. While the articulatory precision was observed in 67.1%, 41.4%, 61.5% and 55.7% of patients during DDK rate of (pa), (ta), (ka) and (pataka) respectively, it was present in all controls.

Discussion and Conclusion: These measures of maximum performance of speech-related tasks can be used clinically to detect deficiencies in the speech motor control in patients with both bulbar and spinal onset patterns of ALS while evaluating for dysarthria.

P125 LARYNGOLOGICAL DETECTION OF THE EARLY BULBAR SIGNS IN LIMB ONSET AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Keywords: videostroboscope,voice, analysis

Background: Not many data concerning the laryngological evaluation of sporadic Amyotrophic Lateral Sclerosis (sALS) patients exist. The bulbar symptoms of sALS include problems with spoken communication (dysphonia, dysarthria), difficulty with the management of swallowing, saliva production and aspiration of the airways. These patients are occasionally seen by otolaryngologists. The first clinical presentation of sALS could be usually on limb or bulbar level only, however the occurrence of preclinical phase of the disease is suggested.

Objective: The aim of the study was to detect the early, preclinical signs of bulbar dysfunction in limb onset sALS patients.

Material and Methods: We have included 24 limb onset sALS patients, diagnosed in the Department of Neurology, Jagiellonian University, Medical College in Krakow; using the El Escorial Criteria (1998). None of patients presented any bulbar symptoms or signs in the neurological examination.

To detect the early bulbar dysfunction the videostroboscope examination with analysis of the acoustic parameters of the voice were performed in sALS cases and results were compared with 20 healthy persons (control group).

Results: In 17 out of 24 patients studied we have noticed-slowness of one / both vocal folds (VF) – (7 patients), the lack of their complete closure during phonation (5 patients) and unilateral decrease of VF tension with limited abduction as well as VF decrease mobility (5 patients).The evidence of shorter phonation time (PT) was detected in 5 out of 24 patients (range: 8 – 18 sec.). The abnormal frequency of the voice (range: 80 – 180 Hz) was detected in 7 patients. The analysis of the Jitter and Shimmer parameters have presented statistical significance difference ($p < 0.001$) as compared to the control group. The voice analysis have showed: roughness voice (5 patients), breathiness voice (5 patients) and asthenicity voice (2 patients). The details of the voice analysis will be presented.

Conclusions: The results of our study have highlighted the occurrence of early signs of bulbar dysfunction, especially nerve/s vagus lesion, before clinical presentation of bulbar failure in limb onset sALS patients. The laryngological examination can be very useful in the estimation of the early bulbar presentation in ALS patients.

P126 VOWEL SPACE AREA IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: vowel triangle, vowel space area, languages

Background: Quadrilateral with the English corner vowels (a), (i), (u) and (ae) has a smaller space area in speakers with Amyotrophic Lateral Sclerosis (ALS) (1). However, (ae) is not observed in the linguistic repertoire of all Indian languages.

Objectives: To determine the vowel space area formed by three vowels in ALS.

Method: 64 patients (Men: Women- 3.92: 1) participated. Among men, 38 had spinal and 13 had bulbar onset. Among women, 7 had spinal and 4 had bulbar onset. They included native speakers of Kannada (15 men, 9 women), Tamil (10 men and 2 women) and Telugu (9 men) languages; and Hindi speakers (17 men, 2 women) of Indo-Aryan background. Age and language compatible 54 healthy volunteers (44 men and 10 women) were the controls.

Words were chosen from the articulation tests of Kannada, Tamil, Telugu and Hindi languages. The language of the articulation test used for participant depended upon his/her linguistic category. Participants either read or repeated words which had the vowels (a), (i) and (u) in predetermined word positions. The first formant frequency (F1-Hertz) and second formant frequency (F2-Hertz) of vowels were obtained using the software- '*speech science lab'*. F1 and F2 values for each of the three vowels of each language were plotted separately graphically for both men and women. A vowel triangle was drawn connecting the plots and the vowel space area (Hertz squared) in the triangle was determined using a mathematical equation and averaged.

Results: For Kannada speaking men, vowel space area was 88,831.37 in spinal onset, 136,122.78 in bulbar onset and 335,098.2 in controls. For Kannada speaking women, area was 276,044.15 in spinal, 160,630.14 in bulbar and 323,360.15 in controls. For Tamil speaking men, area was 90,298.72 in spinal and 144,473.52 in controls. For Tamil speaking women, area was 416,791.95 in spinal and 178,272.37 in controls. For Telugu speaking men, area was 116,683.41 in spinal, 115,274.67 in bulbar and 205,106.44 in controls. For Hindi speaking men, area was 214,348.28 in spinal, 65,179.55 in bulbar and 255,361.89 in controls. For Hindi speaking women, area was 295,737.05 in bulbar and 366,480.35 in controls.

Data suggested that average vowel space area in both spinal and bulbar onset groups, in both genders, is less than area in gender compatible controls irrespective of the language except in one comparison. Bulbar onset groups of Hindi and Telugu speaking men; and Kannada and Hindi speaking women had least area in their respective comparisons.

Discussion and Conclusion: Tongue height and tongue advancement determine the said formant frequencies which in turn determine vowel production. The finding of reduced area in ALS in most of the comparisons suggest inadequate lingual mobility and in turn impaired articulation of vowels and is more so in bulbar onset.

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P127 NOVEL CLINICAL OBSERVATIONS ON POSTURAL ORIENTATION IMPAIRMENTS EARLY IN THE COURSE OF AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Keywords: Balance, Fall, Vistibular impairment

Background: Falls are a common occurrence in ALS patients and may occur before weakness is apparent. Quantification of balance deficit, which may lead to falls Up-and-Gp injuries, is not completely established in ALS patients. Balance deficit may result from damage within the sensory systems (vision, somatosensory, and vestibular) which provide the patient with spatial orientation, from pathology in the central sensory structures necessary for the interpretation and selection of sensory information for balance control, or in the corticospinal tract and associated cortico-bulbar-spinal pathways that permit motor corrections. In ALS clinics, a variety of tests have been used to assess the balance status of ALS patients. The balance subscale of the Tinetti Performance Oriented Mobility Assessment (POMA-B), the Timed Up and Go Test, and the Functional Reach (FR) are among the most frequently used to assess the balance status in ALS patients.

Objectives: To identify balance deficit early in the course ALS.

Methods: We employ the Modified Clinical Test of Sensory Integration and Balance (mCTSIB), in addition to the POMA-B and FR. The mCTSIB requires patient to stand still for 30 seconds on solid ground (1) with eyes open (SG EO) (Three sensory systems available for balance (vision, vestibular, somatosensory) then (2) with eyes closed (SG EC) (vestibular and somatosensory available, vision absent). These two conditions are included in the POMA-B. The patients then are required to stand on foam (3) with eyes open (F EO) (vision and vestibular available, somatosensory compromised) and then (4) with eyes closed (F EC) (vestibular available. vision absent, somatosensory) compromised).

Result: In early diagnosed ALS patients, who are ambulatory without any assistive devices and who report fear of falling (FF) but have not fallen, the mCTSIB is more sensitive to postural imbalance. POMA-B scores were 15 – 16; FR scores were 9 – 15 indicating no apparent risk of falling, but tested patients who tolerated standing on SG EO, SG EC, and F EO with minimal or no sway, developed imbalance with near falling when standing on F EC.

Discussion and Conclusions: Apparent vestibular impairment is indicated early in ALS patients with FF and may not be detected by the commonly used balance scales. Early detection of this impairment should help clinician to develop treatment protocol to rehabilitate this impairment and to teach ALS patients the proper compensation to avoid falls. ALS patients seem to compensate by increasing reliance on vision and somatosensory senses. ALS patients seem to sense this early loss in apparent vestibular system function and compensate by slowing their movement, and assume forward head and trunk posture to increase fixation to maintain the center of mass within the base of support (camptocormia). This strategy may protect patients from falls in the short term, but it may lead to deconditioning of the balance system and the postural righting reflexes needed for proper balance to avoid falls. Further studies of the sensory and motor control of balance in ALS are needed.

P128 CLINICAL PHENOTYPIC ANALYSIS OF X-LINKED RECESSIVE INHERITED SPINAL AND BULBAR MUSCULAR ATROPHY IN CHINA

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Keywords: Kennedy's disease, gene detection, China

Spinal and bulbar muscular atrophy (SBMA) was first described by Kennedy in 1968, and Spada *et al* found that SBMA was caused by an expansion of a CAG repeat in the the first exon of androgen receptor gene. The first family of SBMA confirmed by gene linkage analysis in Chinese mainland was reported in 2006 and other two families reported in 2007. We collected five patients from four families pseudo-diagnosed as SBMA from June to December, 2007. They all have an expansion of a CAG repeat in the androgen receptor gene confirmed by PCR-gene sequencing. This report analyses the clinical phenotype and electrophysiology features of SBMA.

Objective: To analyse the clinical features of X-linked spinal and bulbar muscular atrophy confirmed by gene detection in Chinese.

Methods: Five patients from four families pseudo-diagnosed as SBMA were collected. Four of them have family history. They are all han people from Chinese mainland. Xq$^{11-12}$ androgen receptor (AR) gene was detected by PCR-sequencing directly. Twenty normal Chinese were chosen as the control group. According to the primer sequence designed by La Spada *et al*, a CAG repeat in the the first exon of androgen receptor gene was amplified by PCR. The primer sequence: template strand: 5′2TCCAGAATCTGTTCCAGAGCG2 TGC23′, Crick strand: 5′2GCTGTGAAGGTTGCTGTT-CCT2CAT23′. The symptoms, physical examination of nervous system, electromyogram and creatine phosphokinase were analysed.

Results: The frequencies of CAG repeat in the AR gene's first exon of the five patients were all higher than the control group. The mean frequency of CAG repeat in the control group was 20. In the case group, there were 47 CAG repeats in 1 patient, 50 times in 3 patients and 51 times in 1 patient. Mean age at onset was 27.8 years (15 to 46 years). Some of them were initially diagnosed as low calcium, amyotrophic lateral sclerosis, acquired neuromyotonia, progressive muscula dystrophy. Symptoms of onset were myasthenia of limbs, myospasm, tremor of hands and dysphagia. The slow progressive myasthenia of limbs, especially the lower limbs, was the main symptoms which caused the patients to visit doctor. The second cause was muscle thrilling(3/5), myotonia(3/5). Physical examination: Muscles of tongue amyotrophy, thrill (5/5), bilateral upper extremities weakness (3/5), bilateral lower extremities weakness (5/5), tendon reflex attenuated (4/5), amyotrophy (5/5), amyotrophy of proximate of bilateral upper extremities, thenar, hypothenar and rump(5/5), pathologic reflex and brain stem reflex negative(5/5), waddling gait (3/5), gynecomastia (5/5), giga-potential in electromyogram (5/5). Andrusol and growth were normal and no peripheral nerve disorders. CPK was increased in all patients (mean 496μ/l). one sporadic patient with fewer frequences of CAG repeat suffered milder clinical symptoms, and onset age was later.

Conclusions: The main clinical symptoms of five Chinese patients with SBMA are myasthenia of limbs, especially the lower limbs, and amyotrophy, particularly the upper limb-girdle muscle, muscle spasm, tremor of hands, tendon reflex attenuated mimicking peripheral nerve diseases, waddling gait

and hyper CPK emia mimicking myopathy, giga-potential in electromyogram mimicking anterior horn diseases, gynecomastia without abnormal andrusol and growth.

P129 LATE ONSET BRACHIAL MONOMELIC AMYOTROPHY

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Keywords: Hrayama late onset

Background: Brachial monomelic amyotrophy or Hirayama disease is a benign lower motor neuron disorder affecting mainly young patients, with a male preponderance. Insidious onset of atrophy and weakness, with slow progressive evolution for 2–4 years is followed by a stationary course. Electromyographic evidence of neurogenic pattern without conduction block is typically found.

Objectives: The aim of this work is to present two cases of brachial monomelic amyotrophy of very late onset (63 and 71 years respectively). Previously reported cases of Hirayama disease typically affected young people (98% between 15 and 30 years). To our knowledge no other cases have been published at this advanced age.

Methods: We describe the clinical phenotype of two patients: age of onset, physical activity, duration of progression and clinical manifestations, including initial symptoms and neurological findings during the course of the disease. We also recorded the results of nerve conduction velocity studies, needle electromyography, Anti-GM1 antibody test and cervical magnetic resonance imaging (MRI).

Results: The first patient consulted for progressive left hand weakness appearing at the age of 63. Initially he had difficulty a difficulty making precise movements, without either pain or sensitive symptoms. The neurological examination showed isolated marked left first dorsal interosseous muscle atrophy. Osteotendinous reflexes and sensory examination were normal. Motor and sensory nerve conduction studies were also normal. There were no signs of conduction block. Anti-GM1 antibodies were not present. The symptoms worsened progressively and two years later muscular atrophy had spread to all hand muscles and distal forearm (sparing brachioradialis).

EMG revealed chronic denervation in abductor pollicis brevis, abductor digiti minimi, flexor carpi radialis and first dorsal interosseus on the left side. Cervical spinal cord MRI was normal. From this moment till now, seven years later, there has not been any evolution and the motor deficit remains stable.

The second patient had identical evolution concerning this time the right side: the first sign appeared at the age of 71 and consisted of right first dorsal interosseous atrophy spreading slowly to other hand muscles and distal forearm during a period of two years. There were no conduction blocks and anti-GM1 antibodies were absent. MRI of the spinal cord was normal. He is now 77 years old and the muscular atrophy remains unchanged.

Conclusions: Although brachial monomelic amyotrophy appears typically in young patients, these two cases illustrate that the same phenotype may be seen in older people. Obviously, amyotrophic lateral sclerosis and multifical motor neuropathy must be excluded.

P130 IS SPORADIC MONOMELIC AMYOTROPHY ALWAYS BENIGN?

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Keywords: monomelic amyotrophy, case report, disease progression

Background: Monomelic amyotrophy (MA) of lower limbs is a variant of lower motor neuron disease, generally considered benign and non progressive. We report a case of a MA with a late evolution to a fatal diffuse motor neuron disease (MND).

Case report: A 56 year old caucasian man developed wasting and weakness of the left leg. The patient had no relevant clinical or family history for neuromuscular diseases. The clinical examination revealed signs of lower motor neuron impairment, with amyotrophy, hyporeflexia, fasciculations and cramps, without upper motor neuron signs. Electromyographical (EMG) examination demonstrated a neurogenic pattern, restricted to the left lower limb muscles. Neuroradiological findings (cranial and spinal MRI scan) were negative. Blood chemistry showed normal values, except for a mildly elevated serum creatine kinase (CK). Medial gastrocnemius muscle biopsy was performed: it was suggestive of neurogenic amyotrophy. The disease course was slowly progressive for five years, followed by stabilization of symptoms. However, after a long period (11 years) of disease stability, the contralateral leg was gradually involved, with the appearance of lower motor neuron signs (amyotrophy, weakness and fasciculations). Four years later, both upper limbs, bulbar functions and respiratory muscles were rapidly and severely involved, although there were no evidence of involvement of the upper motor neuron. Within few months the patient died for acute respiratory failure.

Discussion: Our patient's initial clinical presentation had the typical features of lower extremities MA, but he developed a late progression of disease, that turned, 20 years later, to a diffuse motor neuron disease. To our knowledge no other similar cases have been described. This case demonstrated that MA may rarely evolve to a serious form of motor neuron diseases.

P131 CLINICAL AND GENETIC FEATURES OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS IN SOUTH-WEST CHINA

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Keywords: clinical features, prognosis, polymorphism

Background: Amyotrophic lateral sclerosis (ALS) is one of the most common fatal neurodegenerative disorders.

Objective: To investigate the risk and protective factors influencing onset and progression of patients with ALS in South-West China; to analyse the association of several recently reported polymorphisms including rs6587852, rs6700125, rs1470407, rs6690993, rs333662 and rs10493256 and clinical features.

Patients and Methods: All the patients diagnosed with ALS according to the El Escorial criteria from our department from May 2004 to January 2008 were included in the prospected study. Data of clinical features and outcome of

patients were registered. Blood samples were collected after informed consent. The frequency of alleles was identified by sequencing and restriction analysis of the PCR products spanning polymorphism. All the patients were followed up every six months by re-examination or telephone call. Multiple linear regression was used to analyse the prognosis factor of the disease.

Results: Overall 165 patients were diagnosed with definite and probable ALS. The mean onset age was 50.8 ± 11.3 years. In addition, 35 (21.2%) patients were younger than 40 year-old. The number of males (105, 63.6%) was significantly higher than that of females (60, 36.4%).There was no significant difference of onset age, initial symptoms, fasciculation and outcome between males and females. The initial symptom was involved in the upper limbs in 83 cases (50.3%), in the lower limbs in 48 cases (29.1%), in the bulbar in 33 cases (20.0%) and in the respiratory muscles in 1 case (0.6%). The onset age of cases whose initial symptoms involved the spinal cord (132 cases) was significantly younger than that of patients whose initial symptoms involved bulbar (33 cases) (48.9 ± 11.1 vs 58.4 ± 8.4). Forty patients (24.2%) were dead. The onset age of death group (56.7 ± 8.7) was significantly older than that of survival group (48.9 ± 11.4)($p = 0.02$). The mean survival time after onset of disease was 24.3 ± 16.7 months. The mean delay between age at onset and age at diagnosis was significantly shorter in the death group than that in the survival group (12.4 ± 10.8 vs18.3 ± 15.7 months, $p = 0.03$). No association of the polymorphisms and clinical features including onset age, sex, initial symptoms, fasciculation and survival was found in 57 patients group. Positive correlation between mean delay and disease duration was found. Negative correlation between onset age, mean delay and disease duration was found.

Conclusions: Late onset is a poor prognostic factor in ALS patients. Physician should pay more attention to the patients after patients were diagnosed as ALS. No association of the polymorphisms and clinical features may due to the small number of the patients.

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THEME 7 IMAGING, ELECTROPHYSIOLOGY & MARKERS OF DISEASE PROGRESSION

P132 THE UTILITY OF STATISTICAL MOTOR UNIT NUMBER ESTIMATION IN MOTOR NEUROPATHY

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Keywords: motor unit number estimation, demyelination, axonal loss

Introduction: Motor unit number estimation (MUNE) techniques have been applied to the study of muscle denervating disorders to evaluate motor neuron loss. But the features of MUNE in acquired demyelinating neuropathy are not elucidated.

Objective: To compare the features of Statistical motor unit number estimation in amyotrophic lateral sclerosis and acquired demyelinating neuropathy, determine the utility of Statistical MUNE in the differentiating of axonal loss and demyelination.

Methods: 3 groups of subjects including 48 healthy controls, 41 patients with ALS,25 patients with demyelinating neuropathy (19 patients with acute inflammatory demyelinating polyneuropathy, 2 with chronic inflammatory demyelinating polyneuropathy and 4 with motifocal motor neuropathy) were recruited in 2 years. Statistical motor unit number estimations were performed on the median nerve/ thenar muscle. The features of MUNE, the mean amplitude of surface recorded motor unit potential (SMUP), the maximal amplitude of SMUP and the stimulus response function curves in different groups were compared.

Results: The number weighted-MUNE in ALS, demyelinating neuropathy and healthy controls were 49 ± 20, 109 ± 38, 118 ± 12 respectively, the mean amplitude of SMUP were $121 \pm 51\mu V$, $58 \pm 33\mu V$, $110 \pm 25\mu V$ respectively, the maximal amplitude of SMUP were $263 \pm 150\mu V$, $107 \pm 74\mu V$, $139 \pm 36\mu V$ respectively, the amplitude of compound muscle action potential were $5.8 \pm 2.8mV$, $6.5 \pm 4.6mV$, $13.1 \pm 2.8mV$ respectively. There is significant difference between ALS and healthy controls in MUNE, mean amplitude of SMUP and maximal amplitude of SMUP ($p < 0.01$). Mean amplitude of SMUP in demyelinating neuropathy was significantly decreased compared to those in healthy controls and ALS, and there is no difference between demyelinating neuropathy and healthy controls in MUNE and maximal amplitude of SMUP. The range of greatest recording window in stimulating-response function curves increased significantly in ALS.

Conclusions: In patients with ALS, there is a significant decrease in MUNE and increase in maximal amplitude of SMUP; while there is a significant decrease in mean amplitude of SMUP in patients with demyelinating neuropathy, which suggest that Statistical MUNE may be a useful non-invasive technique to help differentiate axonal loss and demyelination.

P133 THE UTILITY OF INCHING TECHNIQUE IN DIAGNOSIS OF MOTOR NERVE CONDUCTION BLOCK

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Keywords: conduction block, inching technique, diagnosis

Introduction: Both motor nerve conduction block (CB) and abnormal temporal dispersion (TD) are electrophysiological events in motor nerve conduction studies. The presence of abnormal TD may interfere in the diagnosis of CB. Motor nerve conduction block is of great importance in differentiating amyotrophic lateral sclerosis (ALS) from acquired demyelinating neuropathy.

Objective: To assess the utility of inching technique in diagnosis of motor nerve conduction block (CB).

Methods: 3 groups of subjects including 30 healthy controls, 44 patients with ALS,38 patients with demyelinating neuropathy (14 patients with inflammatory demyelinating polyradiculoneuropathy, 12 with chronic inflammatory demyelinating polyradiculoneuropathy and 12 with multifocal motor neuropathy) were recruited in 4 years. Routine motor nerve conduction studies (long segment) and inching technique (stimulating along the course of the nerve in 2-cm increments from the wrist to axilla) were performed in median nerves and ulnar nerves simultaneously.

Results: In patients with acquired demyelinating neuropathy, 24 long segments of 18 nerves had a reduction in amplitude of 20% to 40%, no CB or probable CB could be diagnosed according to the criteria of AAEM (1), when studied with inching technique, 21 CBs and 18 probable CBs over short segment were detected in 14 nerves, CBs over short segments were detected with inching technique in 5 long segments with abnormal temporal dispersion which had increase in duration more than 60%. In patients with ALS,No CB over short segment was confirmed in 19 nerves which had a reduction in amplitude of 20% to 40%. According to the criteria recommended by AAEM (1), 4 standard segments of 4 patients with ALS met the criteria of CB or probable CB but not confirmed by inching technique. In all the subjects, no CB over short segment was detected in the long segments with a reduction in amplitude of 20% or less.

Conclusions: Inching technique can give more subtle information of the nerve and have additional diagnostic value in motor neuropathy. Because inching technique was performed with 2 cm interval between stimulating sites, these could decrease phase cancellation and temporal dispersion which is length dependent. Inching technique is more sensitive in detecting CB, useful to extract CB from abnormal TD, and helpful to identify the pseudo-CB in ALS. For segments with a reduction in amplitude of 20% or less, inching technique is not recommended.

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P134 THE VALUE OF SINGLE FIBER ELECTROMYOGRAPHY IN DIAGNOSIS OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: Single fiber electromyography, diagnosis, jitter

Introduction: In the EI Escorial revised criteria for the diagnosis of amyotrophic lateral sclerosis (ALS), single fiber electromyography (SFEMG) is not essential.

Objective: To assess the value of SFEMG in differentiating ALS from other neurogenic disorders.

Methods: SFEMG was performed in extensor digitorum communis muscles (EDC) of 165 patients with ALS and 145 patients with other neurogenetic disorders. The parameters of SFEMG included mean jitter, the percentage of jitter >55μs, the percentage of block and fiber density (FD). Grade scale of the Medical Research Council (MRC) in EDC was assessed simultaneously. The patients were divided into four groups according to the MRC scale (MRC >4 Vs MRC ≤4) and different disease (ALS Vs other neurogenetic disorders). The parameters of SFEMG between different groups were compared.

Results: When the strength of EDC was normal (MRC >4), the mean jitter was (66.1 ± 20.1)μs in ALS and (38.0 ± 9.2)μ s in other neurogenic disorders, P_{50} of the percentage of jitter > 55μs was 55% in ALS and 0 in other neurogenic disorders, P_{50} of the percentage of block was 6.7% in ALS and 0 in other neurogenic disorders, There were significantly difference in those parameters of SFEMG between the two groups($P < 0.01$). When the strength of EDC was abnormal (MRC ≤ 4), the mean jitter was (93.5 ± 31.2)μs in ALS and (52.8 ± 25.9)μ zs in other neurogenic disorders, P_{50} of the percentage of jitter >55μs was 86% in ALS and 20% in other neurogenic disorders, P_{50} of the percentage of block was 20% in ALS and 0 in other neurogenic disorders, There were significantly difference in those parameters of SFEMG between the two groups($p < 0.01$). When SFEMG was performed in EDC with normal muscle strength, the sensitivity and specificity of mean jitter >55μs for diagnosis of ALS were 70.2% and 92.7%. FD was significantly different between ALS and other neurogenic disorders, but the sensitivity and specificity of FD >2.0 for diagnosis of ALS were 87.2% and 31.7% respectively.

Conclusions: The mean jitter, the percentage of jitter >55μs and block are of great importance in differentiating ALS from other neurogenic disorders in the early stage. When SFEMG is studied for early diagnosis of ALS, it is recommended to perform in muscles with normal strength. If jitter increases significantly with block and increased FD in muscles with normal strength, it supports the diagnosis of ALS. If mean jitter and/or FD are within normal limits, it is inconsistent with the diagnosis of ALS and should suggest the presence of other disease.

P135 COMPARISON OF MUNE AND CLINICAL MEASURES IN PATIENTS WITH ALS

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Keywords: MUNE, ALSFRS, CMAP

Background: Motor unit number estimation (MUNE) has been developed to elucidate the pathophysiologic features of axonal loss in ALS. MUNE has been used as endpoint measures in clinical trials for drug studies in ALS. Multiple point stimulation MUNE has good test–retest reliability and can be used on any EMG machine. The advantage of MUNE must be compared to other more familiar metrics such as CMAP, muscle strength and ALSFRS-R.

Objectives: This study aims to evaluate the correlation between neurophysiological measures and clinical functional scales in patients with ALS.

Methods: The subjects of the study included 23 patients with ALS. CMAP and MUNE were recorded from the ADM by stimulating the ulnar nerve. The automated multiple point stimulation method was used for MUNE. MUNE was tested twice by two experienced examiners, and averaged data were adopted as the results. The grip power, ALSFRS and Norris scale were also measured simultaneously to assess the physical functions. In addition to the total scores of ALSFRS (ALSFRS-T) and Norris scale (Norris-T), we applied the sub-scores relating to the hand functions as ALSFRS-H and Norris-H. Both MUNE and CMAP data were compared with the grip power, ALSFRS and Norris scale. Spearman's rank-order correlation coefficient was used for statistical analysis of clinical scales.

Results: MUNE was well correlated with ALSFRS-H ($p = 0.723$), Norris-T ($p = 0.654$) and Norris-H ($p = 0.846$) significantly. The correlation of MUNE with ALSFRS-T ($p = 0.350$) was not significant. On the other hand, CMAP was correlated significantly with Norris-H ($p = 0.812$) and ALSFRS-H ($p = 0.730$). The correlation of CMAP between ALSFRS-T ($p = 0.330$), and Norris-T ($p = 0.613$) was not significant. The grip power had linear correlation with MUNE ($r = 0.811$) and CMAP ($r = 0.817$) significantly. In some cases at the early stage of ALS, MUNE showed some decline, although both ALSFRS and Norris score were nearly full marks.

Conclusion: MUNE and CMAP were well correlated with the physical functions equally. MUNE was more sensitive than those functional scales at the early stage or pre-clinical stage of ALS, but CMAP was also good as the endpoint measure in the advanced stage.

P136 CONTACT HEAT EVOKED POTENTIALS IN PATIENTS WITH ALS

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Keywords: pain pathway, contact heat evoked potentials

Objectives: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease involving both upper and lower motor neurons while the sensory system is spared. The purpose of this study is to investigate the characteristics of contact heat evoked potentials (CHEP) in patients with amyotrophic lateral sclerosis (ALS) and evaluate the nociceptive pathway in these patients.

Methods: Sixty cases of definite ALS patients were diagnosed according to the criteria of El Escorial. All patients and sixty healthy controls had pain elicited by a CHEP stimulator with a diameter of 27mm (area 573 mm^2) and an accelerated velocity of 70°C/s. Thermal stimuli were sent at 54.5°C to three body sites: the dorsum of the hand, proximal volar forearm and C7. CHEP was recorded using a Keypoint.net (Medtronic, Skovlunde, Denmark) from midline electrodes (Cz and Pz positions of the standard 10–20 EEG montage. At same time, somaosensory evoked potential (SEP) was performed.

Results: The three mainly described pain evoked potential components were visible at 54.5°C which was a painful level to the subjects. These were Cz/N550, Cz/P750 and Pz/P1000. We measured the peak latency of the main negative components. The waveforms and latencies of CHEP in ALS patients were normal. The latencies were 561.2 ± 28.6 ms at the dorsum of the hand, 540.1 ± 39.2 ms at the proximal volar forearm and 512.7 ± 31.4 ms at C7. There were no significant differences between the ALS patients and the healthy controls in CHEP ($t = 4.23, 4.51, 3.74$, $p > 0.05$) or SEP.

Conclusions: CHEP in patients with ALS is normal, which suggests that the nociceptive pathway is intact. This result supports the idea that ALS selectively involves the motor system and that sensory system disturbances are rare.

P137 MODELLING NEUROLOGICAL DECLINE IN ALS PATIENTS

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Keywords: MUNE, Markov death process, Monte Carlo Markov Chain (MCMC)

Background: In order to better understand the mechanisms whereby neurodegenerative diseases such as ALS advance, a number of models have been put forward to describe the declining numbers of surviving motor neurons. The simplest of these models is the 'one hit model'. Here the neurons are assumed independent and subject to a fixed risk of a catastrophic biochemical event leading to an exponential decline in the number of surviving neurons.

Objectives: We present in this paper a methodology for evaluating the evidence in favour of the exponential model against a more general model in which the probability of death of a neuron is allowed to vary over the course of the disease.

Methodology: We use a two-stage procedure. The first stage involves the analysis of a surface electromyographical scan carried out on several occasions over a period of time in the course of the disease. The method of Ridall *et al.* (2006) is used to arrive at an estimate of the number of remaining motor units supplying a given muscle. The method makes use of reversible jump Markov chain Monte Carlo (RJMCMC(Green 1995), a computationally intensive method in Bayesian inference. The second stage involves the analysis of the RJMCMC output using a hidden stochastic death process. Two models are compared. In the first, the rate parameter is assumed fixed (corresponding to exponential decline), whereas the second allows the rate parameter to evolve over time. The evidence in favour of each of these models is compared by using a Bayes factor.

Results: In eight of the ten ALS subjects studied, we found that the evidence favoured the one hit model, implying that the decline is exponential. For two of the patients the exponential model is inadequate and there is strong evidence suggesting that the rate of decline is not constant.

Discussion: For a majority of the patients studied the decline of surviving motor units can be taken to be exponential and the rate of onset of the disease can be summarised by a credible interval representing the half life of a typical neuron. Such an estimate can be obtained using a programmed electromyographical scan and using software available from the authors. For these patients we can make two conclusions. The first is that the probability of death of a unit does not seem to be related to the number of dying or dead motor units Second, the motor units in question appear to have a similar hazard of death. For two of the patients the exponential model appears inadequate to describe the decline, suggesting that the above conclusions cannot be made.

P138 ISOLATED CONTINUOUS RHYTHMIC LINGUAL MYOCLONUS: UNUSUAL PRESENTATION OF AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: Lingual Myoclonus; Bulbar onset.

Introduction: Isolated myoclonus of the tongue is an exceptional entity, poorly documented and understood. Recently we observed a case of isolated continuous rhythmic lingual myoclonus as first symptom of amyotrophic lateral sclerosis (ALS).

Case Description: A 45-year-old woman suddenly manifested a continuous involuntary movement of tongue and dysarthric speech. Neurological examination revealed continuous, rhythmic bilateral symmetric jerks of the tongue that produced a narrowing of its anterior portion, with a slight forward protrusion and without lateral deviation. The movements were continuous, apparently rhythmic, when the tongue was protruded and when it was at rest. No other branchial muscles involvement was observed. At the onset no other neurological signs were manifested. EEG, BAEP SEPs and brain MR were normal. EMG recorded from the bilateral genioglossus muscle showed continuous, low-frequency (1–2 Hz), rhythmic bursts of bilaterally synchronous muscle activity. Six months later she developed a progressive bulbar and motor palsy typical of ALS.

Conclusions: To our knowledge this represents the first report of isolated continuous rhythmic lingual myoclonus as first sign of ALS. Imbalance of excitatory neurotransmission as reported at the pre-clinical phase of bulbar-ALS could explain this rare form of myoclonus.

P139 ALS AND TMS: TRAPEZIUS MUSCLE EVALUATION

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Keywords: TMS, Central Motor Conduction Time, Trapezius muscle

Background: Transcranial magnetic stimulation(TMS) is a useful and safe method to test the corticospinal system integrity. In ALS the early detection of the upper motor neuron(UMN) involvement represents an important marker of the disease.

Objectives: We applied TMS in a sample of consecutive ALS.

Methods: We studied 38 ALS patients (23 males; 54 ± 13 yrs; spinal onset, $n = 28$). Based on the El Escorial criteria we had 18 clinically definite, 17 clinically probable and 3 clinically possible. With a large round coil over the vertex we tested the bilateral trapezius, first dorsal interosseous(FDI) and tibialis anterior(TA) muscles. For all we determined the relaxed

threshold, the total, peripheral and central motor conduction time(CMCT). We compared ALS data with a group of 25 normal controls.

Results: Average relaxed threshold was increased in the ALS group(54.0±13.5 vs 47.6±6.0; p=0,01). In ALS patients the CMCT was prolonged: in 80% of the cases when based on trapezius recordings; in 66% of the cases when based on TA and in 48% of the cases when based on FDI recordings. Considering all muscles, the CMCT was impaired in 98% of patients. Considering trapezius and TA recordings, the pathological CMCT emerged in 92% of the cases. Although we had only 3 patients with clinically possible ALS, trapezius CMCTs were prolonged in all of them, while were normal for the FDI.

Conclusions: TMS evaluation of the trapezius muscle can represent a promising target of study. It can be expected to be able to distinguish ALS from cervical spondylotic myelopathy, and to detect the early involvement of UMN. The combined evaluation of trapezius and TA muscles appears to increase this probability

P140 ALS AND TMS: CENTRAL SILENT PERIOD

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Keywords: TMS; Central silent Period

Background: Suppression of the volitional EMG activity due to a transcranial magnetic stimulus (TMS) is called central silent period (CSP). In amyotrophic lateral sclerosis (ALS) CSP was usually reported reduced in length.

Objectives: To study the CSP in a sample of consecutive ALS.

Methods: We studied 38 ALS patients (23 males; men age 54±13; spinal form=28). Based on the El Escorial criteria we had 18 clinically definite; 17 clinically probable and 3 clinically possible. TMS was delivered with a large round coil over the vertex; intensity of stimulation was set=1.5x relaxed threshold. Motor evoked potentials and CSP were recorded bilaterally from the first dorsal interosseous muscle (FDI) while patients performed their maximum effort. For each muscle we recorded at least eight trails. We correlated the CSP duration with clinical variables. We compared data of ALS patients with a group of 25 normal controls.

Results: In patients, the CSP duration was not different as compared with the controls (199.9 ms vs 185.8 ms), while the standard deviation was much higher (±69.7 ms vs ±20.5 ms). A cluster analysis defined three CSP homogeneous groups, termed "short CSP", mean value=135 ms, which was the most frequent (42.7%); "normal"(mean value 218 ms; 40.3%), and "long" (mean value 311 ms; 17%). The CSP length was significantly correlated to the Norris score(p=0.025) and to the Babinski sign(p=0.047), while did not correlate with the ALS duration.

Conclusions: In our patients, the CSP showed a heterogeneous pattern. The most frequent was represented by a "short" CSP, although "normal" and "long" durations were not so infrequent. CSP length was correlated with ALS clinical severity.

P141 EFFECT OF TRANSCRANIAL DIRECT CURRENT STIMULATION ON THE CORTICAL EXCITABILITY IN PATIENTS WITH ALS

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Keywords: cortical excitability, transcranial direct current stimulation, transcranial magnetic stimulation

Background: Although the exact aetiology of ALS is unclear, hyper excitability of cortical cells is accepted as a component of the neuronal degeneration in ALS. Cathodal transcranial direct current stimulation (tDCS) is a tool to reduce the cortical excitability. In patients with ALS only one study of tDCS is performed (1). They used a single protocol where anodal/cathodal tDCS was performed for 7 minutes in ALS patients and healthy controls. They were able to induce a consistent modification of cortical excitability in healthy subjects but not in patients with ALS.

Objectives: To investigate the differences in motor cortex excitability between patients with ALS and healthy controls. To investigate the effects of different tDCS stimulation durations on the motor cortex excitability in patients with ALS compared to healthy controls.

Methods: Cathodal tDCS stimulation in 3 random sessions (7, 11 and 15 minutes), was performed on both 10 healthy controls and 10 patients with ALS. The primary outcome measure was cortical excitability measured by transcranial magnetic stimulation as resting motor threshold (RMT), single-pulse motor evoked potentials (single MEP) at a stimulus intensity producing MEPs with mean amplitudes of 1mV at baseline, intracortical inhibition (ICI) and facilitation (ICF). For the evaluation of the impact of tDCS these TMS indices were measured: before tDCS (pre), 5 and 20 minutes after the tDCS (post 1 and 2).

Results: No significant differences were found in motor cortex excitability between patients with ALS and healthy controls, prior to the tDCS.

Healthy controls showed a significant inhibition of the single MEP amplitudes and an increased RMT after 15 min of tDCS. In the patients, as a group, there was no significant inhibition of the single MEP amplitudes and no significant increase of the RMT after 15 minutes of tDCS. However, of the 10 ALS patients, 6 showed a response of cortical inhibition that resembled those of the healthy controls. The paired pulse results showed an exaggerated, not significant ICF effect in healthy controls, in all sessions. The ICI and ICF were not affected in the ALS patients after tDCS. Repeated measures ANOVA on single MEP 1mV and RMT did reveal a main effect on time, not on group.

Discussion and Conclusion: tDCS is able to induce a duration dependent cortical inhibition in healthy controls. Intriguingly, tDCS induced an exaggerated ICF effect in these healthy subjects. In patients with ALS, the effect of tDCS on cortical inhibition shows much more variation between individuals. For that, we explored the causes of this difference in response to tDCS. Age and disease duration showed no relation with the response to tDCS.

Reference:

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P142 EVALUATION OF CHANGES OF UPPER MOTOR NEURON IN ALS WITH DTI AND TRANSCRANIAL MAGNETIC STIMULATION MOTOR EVOKED POTENTIALS

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Keywords: UMN, CMCT, FA

Background: Amyotrophic lateral sclerosis (ALS) is selectively characterized by lower motor neuron (LMN) signs of limb and bulbar muscles accompanying with upper motor neuron (UMN) signs. Subclinical LMN involvement is detectable by electromyographic findings of denervation. However, UMN involvement can be evaluated by physiologic and clinical measures without any objective diagnostic criteria.

Objective: To investigate the diffusion tensor imaging (DTI) measures and transcranial magnetic stimulation motor evoked potential (TMS-MEP) changes in ALS and their correlations with clinical features. To assess the value of DTI measures and TMS-MEP in the diagnosis UMN involvement in ALS.

Methods: 35 ALS patients complying with the revised El Escorial criteria and 30 age and sex matched healthy controls were enrolled in the study. We measured central motor conduction times (CMCT) for the bilateral abductor digiti minimi(ADM) and tibialis anterior (TA) muscles by TMS-MEP. At the same time, we investigated 11 patients with ALS from above and 10 healthy controls by DTI and measured fractional anisotropy (FA) within the corticospinal tracts and in the extramotor white matter. Clinical status of each patient was evaluated with the ALS Functional Rating Scale–Revised (ALSFRS-R) and UMN involvements were assessed by Ellis reflex scale.

Results: The abnormalities of the ALS patients measured by TMS-MEP were about 65%. The manifestations were prolonged CMCT and absent MEP, which increased significantly compared with controls. CMCT correlated significantly with Ellis scales and ALSFRS-R. There was a significant decrease of mean FA in all regions of the corticospinal tract in patients with ALS as compared with controls. FA along the corticospinal tract decreased significantly with higher UMN scores.

Conclusions: TMS-MEP could find UMN involvement in the ALS patients, but it is not sensitive for early diagnosis. The changes of CMCT correlated with the degree of UMN involvement. FA reflects functional abnormality of intracranial corticospinal tracts and can be used for objective evaluation of UMN involvement in ALS. Combining CMCT and FA may be a better index for early diagnosis UMN involvement in ALS patients.

P143 A ^{1}H-MRS STUDY OF THE PRECENTRAL GYRUS IN ALS: CORRELATION WITH CLINICAL FINDINGS

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Keywords: 1H-MRS, marker, precentral gyrus

Objectives: To explore the significance of ^{1}H-MRS on studying amyotrophic lateral sclerosis (ALS), hoping to find surrogate markers useful for diagnosing ALS, for quantifying the degree of upper motor neuron (UMN) degeneration and for monitoring disease severity and activity.

Methods: Ninety healthy controls, 110 patients with ALS and 24 patients with lower motor neuron syndrome (LMNS) underwent structural MRI and single-voxel ^{1}H-MRS. Measurements of the metabolic ratios NAA/Cr, Cho/Cr and NAA/Cho were compared and correlated with clinical assessments.

Results: The Pearson correlation coefficient was 0.823 for NAA/Cr and 0.712 for Cho/Cr. The 3 metabolic ratios in the precentral gyrus had great ranges in healthy adults, and had no lateral and sexual differences and correlated with aging (Pearson correlation coefficients were –0.220 for NAA/Cr, 0.240 for Cho/Cr and –0.464 for NAA/Cho). When compared with controls, NAA/Cr in total ALS patients and definite ALS patients decreased significantly; NAA/Cr in patients with probable and possible ALS and with LMNS did not change significantly, and Cho/Cr of total ALS patients increased significantly. NAA/Cr in patients with definite ALS was lower than those of patients with probable and possible ALS. No significant differences in metabolite ratios were found within probable ALS, possible ALS and LMNS patients, and the same between the patients with bulbar onset and limb onset between the sides with onset and no onset and between the patients with CST hyperintensity and not. NAA/Cr of patients with definite UMN signs was lower significantly than that of patients with probable UMN signs. We also found the NAA/Cr of corresponding precentral gyrus of more severe clinical manifest was lower statistically than that of the other side. Correlation analyses revealed the following: (1) the 3 metabolite ratios NAA/Cr, Cho/Cr and NAA/Cho all had no correlation with disease duration; (2) NAA/Cr and Cho/Cr were correlated with reflex score, while NAA/Cho were not; (3) NAA/Cr was correlated with ALS-FRS, APPEL total score and all sub-items of APPEL (Pearson correlation coeffetients differed from -0.251 to 0.457).

Conclusions: ^{1}H-MRS was useful to study ALS. The marker NAA/Cr of precentral gyrus might reflect UMN degeneration degree, disease severity and disease progression of ALS patients, while it was not helpful to early diagnosis.

P144 NEUROCHEMICAL ABNORMALITIES IN THE PREFRONTAL CORTEX OF PATIENTS WITH ALS/MND

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Keywords: magnetic resonance spectroscopy, cognitive impairment

Background: ALS/MND is a heterogeneous disorder and not a pure motor neuron disease. Nearly 50% of patients have some degree of cognitive impairment due to frontotemporal lobar degeneration. A better understanding of the biological basis of cognitive impairment would provide insight into varying pathogenic mechanisms associated with clinical subtypes. Magnetic resonance spectroscopy (MRS) permits in vivo of measurement of metabolites relevant to several aspects of cerebral degeneration, including neuronal and astrocytic density, and neurotransmitter metabolism.

Objectives: To study prefrontal cortex (PFC) neurochemistry and its association with cognitive impairment and disability in ALS.

Methods: High field MRS was performed in the PFC in patients with definite or probable ALS (El Escorial criteria) and controls. Sequences implementing double quantum filtration and spectrally selective refocusing were used to

measure the neurotransmitters glutamate and gamma-amino-butyric acid (GABA) as well as their intermediary metabolite glutamine, the neuronal marker *N*-acetylaspartate (NAA), and the astrocytic marker *myo*-inositol. Subjects underwent cognitive and behavioural evaluations. Thirteen patients with ALS and 11 healthy controls have been studied to date.

Results: Comparing ALS to control subjects, *myo*-inositol was increased (6.04 ± 0.9 vs 5.10 ± 0.9, $p = 0.03$). A reduction in NAA approached statistical significance (9.13 ± 1.6 vs 10.2 ± 1.8, $p = 0.09$). Glutamate, glutamine, and GABA were unchanged. PFC neurochemicals did not correlate with cognitive measures. However, a correlation was found between glutamate and the ALSFRS-R ($r = -0.66$, $p = 0.02$).

Discussion and Conclusions: Cerebral neurochemical abnormalities exist beyond the motor cortex in ALS and correlate with disability. PFC neurochemistry profiling with MRS may provide a biomarker for global impairment in ALS.

P145 BRAIN ENDOPHENOTYPE OF BULBAR AND LIMB ONSET ALS IN DIFFUSION TENSOR IMAGING

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Keywords: endophenotype, diffusion, diagnosis

Background: In ALS, recent MRI voxel based analyses have found widespread cortical atrophy, and white matter changes along the corticospinal tract (CST) and in frontal and transcallosal projections. It is as yet unclear whether extremity or bulbar onset ALS display different brain endophenotypes.

O**bjectives:** To characterize diffusion alterations in bulbar and limb onset ALS patients brains by whole brain voxel based MRI analyses.

Methods: We examined 17 mildly affected (ALSFRS ~ 40) ALS patients and 17 age-matched controls in a GE 1.5 T scanner. Diffusion tensor image sets were generated from 12 planes with eddy current correction. The apparent diffusion coefficient (ADC) and fractional anisotropy (FA) were analyzed in group comparisons and correlated to clinical parameters using SPM software.

Results: All ALS patients showed a significant reduction in FA along the corticospinal tract (CST) compared to controls. ADC images sets, however, were more sensitive in detecting these changes, and showed abnormal diffusion in the white matter extending into frontal regions. In extremity onset ALS only frontal white matter ADC abnormalities were found, whereas in bulbar ALS, the CST and adjacent areas were primarily affected.

Conclusions: This in-vivo neuropatholoy MRI study sheds light on the distribution pattern of diffusion abnormalities of different onset type ALS. It raises the question whether these subtypes are caused by specific degenerative processes.

P146 CORTICAL ACTIVATION IN ALS PATIENTS WITH/WITHOUT BULBAR SIGNS: AN FMRI-STUDY

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Keywords: fMRI, cortical activation, bulbar signs

Objective: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder of the upper and lower motor neurons with a progressive limbic or bulbar muscular weakness and wasting. Survival of patients with bulbar onset is known to be shorter than of patients with lumbar onset. In this study we investigated the cortical activity during movements of tongue, eyes and extremities with fMRI.

Methods: We studied 18 patients with ALS and 20 healthy controls, using BOLD-fMRI, while they performed an acoustically paced motor task. fMRI data were analyzed with Brain Voyager QX.

Results: Task-related functional activations were identified in motor cortical regions in both patients and healthy controls. There were no differences between the two groups during horizontal eye movements. The movements of tongue in ALS patients caused less activation, especially in subcortical areas, in contrast to healthy controls. However, the motor activity of extremities showed increased cortical activation in the ALS group.

Conclusions: This data demonstrates a different activation mode in ALS patients in contrast to healthy controls. For the first time a different activity pattern between ALS patients and healthy controls during vertical tongue movements could be demonstrated.

P147 IRON DEPOSITS IN ALS AS EVALUATED BY MRI COMBINED WITH FREE RADICAL ASSESSMENT USING EPR PRELIMINARY DATA

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Keywords: amyotrophic lateral sclerosis, magnetic resonance imagining, EPR spectroscopy

Background: It has been suggested that iron-dependent MRI contrast in the brain may provide a new biomarker of the presence and progression of a variety neurodegenerative disorders such as Parkinson's and Alzheimer's disease. The results of the MRI studies were, in one way, different in ALS. Hypointesity of the precentral gray matter (PGGM) in T2W MRI was reported in ALS patients in several studies. Although it was presumed that PGGM were iron deposits, more elaborate MRI techniques are needed. There are not many data available about free radical production capacity of CSF, neither data about the possible role of iron oxidative stress in ALS.

Objectives: The aim of the study was to investigate the MRI sequence as a potential marker for iron and to establish the possible connection between PGGM on MRI in ALS patients and free radical production, by measuring free radical capacity of CSF using EPR spectroscopy.

Methods: Thirty five (24 female and 11 male) newly diagnosed SALS patients aged 56 ± 9 (mean $\pm$ SD.) (Range 39–74) years with probable or definite ALS according to El Escorial criteria were included in this study. Nine patients had a bulbar onset and 26 patients limb onset of the disease. The mean duration since onset of symptoms, was 14.2 ± 6 months. The ALSFRS-R was performed in all patients before MRI. We used a routine MRI protocol, including FLAIR, but we also added a thin slice T2*W gradient echo technique targeted for detection of iron deposits. The PGGM score was assessed for the presence of hypointensity (graded 0–3). Samples of CSF represented remains of CSF obtained for routine medical purpose from 20 SALS patients. Control group comprised eleven age- and sex-matched controls. H_2O_2 was added to induce free radical production and samples were measured with the addition of DEMPRO spin trap using standard EPR spectrometer.

Results: The mean value of ALSFRS-R score was 35 ± 9 points. We found a statistically significant correlation between ALSFRS score and MRI score ($r = 0.69$). The production of free radicals in the CSF was greatly enhanced in ALS patients reaching 4 times values in comparison to control patients. The correlation between EPR and MRI score was ($r = 0.49$).

Conclusion: The results of this study showed that the use of T2*W sequence was more efficient than either FLAIR or conventional T2W in detecting iron deposits in precentral gyruses due its sensitivity to iron induced susceptibility artifacts. EPR measurement of free radicals in CSF pointed out to increased total production of free radicals in CSF of ALS patients mostly like through Fenton reaction, which potentially links these findings to iron deposits found in the brain.

P148 MITOCHONDRIAL PERIPHERAL BENZODIAZEPINE RECEPTOR IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: mitochondria, mitochondrial permeability transition pore, peripheral benzodiazepine receptor

Background: Amyotrophic Lateral Sclerosis (ALS) is a degenerative disease of unknown origin characterized by the progressive loss of motor neurons of the anterior horns in the cortex, bulb and spinal cord. Among the several pathogenetic mechanisms considered in the attempt to explain motor neuron death in ALS, the hypothesis of the occurrence of mitochondrial dysfunction has received considerable interest. Permeabilization of mitochondrial membranes, due to the opening of the mitochondrial permeability transition pore (MPTP), is considered an important step in the apoptotic or necrotic cell death cascade. MPTP is a multiprotein complex in which the peripheral benzodiazepine receptor (PBR) represents a core structural protein. PBR has been recently reported to play an important role in the regulation of MPT and in the control of apoptotic cell death.

Objectives: to evaluate the kinetic binding parameters of the specific PBR ligand, PK 11195, in platelets obtained from patients affected by ALS.

Methods: 16 ALS patients (mean 64.1 ± 2.39 years) and 18 control healthy volunteers (mean, 40.3 ± 3.06 years) were recruited for the study.

Platelets obtained from each ALS patient and healthy volunteer were centrifuged and stored at -80°C. Aliquots of platelet membrane suspensions were incubated with increasing concentrations of [3H] PK 11195, either in the absence (total binding) or in the presence (non-specific binding) of PK 11195. After incubation, the samples were filtered, washed and the radioactivity was counted, using a liquid-phase scintillation counter.

Results: The PBR kinetic binding parameters in platelet membranes from ALS and controls were saturable and with high affinity. Scatchard plots were linear for all analysed subjects, suggesting the presence of a homogeneous population of binding sites. A significant decrease in PBR Bmax values was observed in platelets of ALS patient as compared to controls ($p = 0.002$), whereas the Kd values did not differ significantly. The relationship between the reduction of platelet PBR Bmax and the disease progression showed a significant correlation ($r = 0.62$, $p < 0.01$).

Conclusion: The present data are consistent with an alteration of PBR density in ALS patients. This indicates a possible role of PBR in the pathogenesis of ALS and, at the same time, suggests a perspective use of it as candidate biomarker to trace disease severity and progression.

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THEME 8 COGNITIVE AND PSYCHOLOGICAL ASSESSMENT AND SUPPORT

P149 AN I113T MUTATION IN THE SOD-1 GENE ASSOCIATED WITH SEVERE FRONTOTEMPORAL DEMENTIA IN A PATIENT WITH FAMILIAL ALS

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Keywords: SOD-1 mutation, frontotemporal dementia, primary progressive aphesia

Background: Mutations that cause amyotrophic lateral sclerosis/frontotemporal dementia have been found in genes for microtubular associated protein tau-2 (MAPT-2), progranulin (PRG), the valosin containing protein gene (VCP) and in the dynactin 1 gene (DCTN1). While SOD-1 is the most common form of familial ALS, there is little evidence that this mutation can also cause dementia. To date, there have been only two cases of FTD reported with SOD-1 mutations. In one the mutation was G41S and in the other leu144phe. Neuropsychological testing and PET studies have also been found to show abnormalities in some patients with SOD-1 mutations who do not have frank dementia.

Objective: To present a patient with an I113T mutation in the SOD-1 gene, who developed progressive and ultimately severe FTD two years before the onset of motor weakness and ALS.

Methods: A 54 year old man developed progressive difficulty with language and behavior. The family thought his behavior had become withdrawn. Gradually, he also began to have progressive difficulty with grammar and word finding and became more disinhibited in his behavior. Two years after onset he began to develop weakness. At the time of presentation to our center, three years after onset, he was essentially mute and had difficulty following even one step commands. He could write simple letters but not words and perseverated in responding to commands. He had bilateral hand and ankle weakness. Reflexes were brisk in all four limbs. There was no obvious involvement of bulbar function, with no tongue involvement, suggesting his speech difficulty was purely the result of aphasia.

Family history was significant for ALS in his father, paternal uncle and two of his cousins. His father, who had died years earlier, was thought to have unusual behavioral changes during the last few years of his life, where he was reported as wearing outlandish outfits and drinking excessively for the first time in his life.

Results: Genetic testing revealed an I113T mutation in the SOD-1 gene.

Conclusions: The I113T mutation is the most common SOD-1 mutation in some European countries, but has not yet been associated with dementia. Our case provides further evidence that a variety of SOD-1 mutations can be associated with classic FTD, albeit with very low penetrance compared to the motor phenotype. While this is a single family, the finding is important because it offers insight into the causative mechanisms and relationship between the two diseases.

P150 EYE MOVEMENT PROFILE IN MOTOR NEURONE DISEASE

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Keywords: Ocular fixation, Saccades, Smooth pursuit

Objective: Studies of eye movements in Motor Neurone Disease (MND) to date have been conflicting and studies have been small. In addition, ocular fixation has not yet been formally examined. With the recent characterization of ocular fixation using saccadic intrusion (SI) amplitude and fixation periods, we performed a cross-sectional observational study to examine ocular fixation, saccades and pursuit eye movements in patients with MND.

Methods: Forty-four patients and forty-five controls were recruited. Ocular fixation, reflexive saccades, antisaccades and smooth pursuit were examined using infra-red oculography. All subjects then underwent a neuropsychological evaluation.

Results: SI amplitude ($p = 0.01$), antisaccade latency ($p = 0.01$) and antisaccade type 1 errors ($p = 0.03$, $p = 0.04$) were increased and smooth pursuit gain was reduced ($p = 0.00$) in patients compared to controls. SI amplitude, antisaccade errors and smooth pursuit gain correlated with neuropsychological measures sensitive to lesions of the frontal lobes.

Conclusions: These results indicate that abnormalities in ocular fixation, saccades and smooth pursuit may be a marker of the sub-clinical frontal lobe dysfunction in MND. A longitudinal study to examine if these measurements deteriorate with time would be of great interest as this could provide a quantifiable objective marker of disease progression.

P151 CHANGES IN BEHAVIOUR AND SOCIAL COGNITION IN AMYOTROPIC LATERAL SCLEROSIS WITHOUT DEMENTIA

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Keywords: cogniton, abherrant-behaviour, orbitomedial-prefrontal-cortex

Background: Executive dysfunction, associated with dysfunction of dorsolateral prefrontal cortex (DLPFC), has been demonstrated in up to 60% of individuals with ALS. Reports of personality and behavioural changes affecting daily life, in combination with evidence of pathological alterations, suggest functions of the orbital and medial prefrontal cortex (OMPFC) may also be impaired early in the disease course.

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Objective: To assess social cognition and behavioural change in individuals with ALS without dementia, using neuropsychological tests known to be sensitive to OMPFC dysfunction.

Methods: Participants comprised of 16 non-demented ALS patients, 16 informants (spouse or family member) and 16 controls matched for age, education, ethnicity and gender. Forced vital capacity (FVC) and disability measured by the ALSFRS-R were recorded for patients; anxiety and depression were assessed by the Hospital Anxiety and Depression Scale. OMPFC function was assessed via a theory of mind task (Faux Pas Test), recognition of emotional prosody (Aprosodia Battery subtest), decision making (Apartment Task), response to changes in environmental reinforcement (Probabilistic Reversal Learning), and behavioural change (NPI administered to informants). DLPFC function was assessed in the areas of cognitive flexibility (oral and written letter and category fluency), planning (Stockings of Cambridge, SOC) and self and informant report of executive functioning (BRIEF-A). Analyses were completed using the Students t-test, repeated measures ANOVA, the Mann-Whitney U test and z-scores.

Results: There were no significant differences between groups for age, years of education, gender, anxiety or depression. On tasks sensitive to OMPFC dysfunction, the ALS group had significantly lower scores on the Aprosodia subtest and the Faux Pas Test, but not the Apartment or Reversal Learning tasks. Aberrant behaviours on the NPI for the ALS group were agitation, apathy, disinhibition, stereotypic behaviour, sleep disturbance and irritability. On tasks sensitive to DLPFC function, the ALS group had significantly lower scores for oral fluency (letter W), mean correct solutions for SOC easy trials (but not hard trials) and time to correct solution for SOC hard trials (but not easy trials). Significant differences were also found between ALS self and informant BRIEF-A group scores for the planning/organisation and organisation of materials subscales and the meta-cognition index, and between ALS self and control BRIEF-A group subscale scores for organisation of materials and the meta-cognition index. In both cases the ALS group rated their performance as better. Individually, 61% of the ALS group had impaired OMPFC function, 46% had impaired DLPFC function, and 31% had no impairment (based on z-scores < -2 or scores outside control range).

Conclusions: OMPFC dysfunction was demonstrated in ALS with group and/or individual impairments in emotional prosody recognition, Theory of Mind, decision making, reversal learning and presence of aberrant behaviours. Individual analysis indicated deficits associated with OMPFC dysfunction are at least as common as DLPFC dysfunction. Due to the particular stresses put on carers and family members by the type of impairments related to dysfunction of OMPFC, we believe detection of such difficulties should be an important clinical priority.

P152 EXECUTIVE DYSFUNCTION IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: ALS, cognitive impairment, executive dysfunction

Background: Neuropsychological investigations of amyotrophic lateral sclerosis (ALS) patients have revealed cognitive impairment in some non demented patients, characterised mostly by executive dysfunction. The most striking and consistent deficits were found using tests of verbal fluency.

Objectives: To investigate the profile of executive dysfunction in non-demented patients with ALS.

Methods: Forty two non demented patients with SALS (23 females, 19 males) with probable or definite ALS according to El Escorial criteria were included in this study. Control group comprised of 18 age matched healthy controls. The duration of disease since onset of symptoms, was 18 months. The battery of neuropsychological tests, designed to accommodate the range of physical disability present in ALS, included: Mini Mental State Test; Hamilton Rating Scale for Depression; Scale of Apathy; Test Raven Progressive Matrix; Letter Fluency Test; Category Fluency Test (Animal Naming Test); Boston Naming Test and Cambridge Neuropsychological Test Automated Battery (CANTAB), including: Motor Screening, Spatial Span, Spatial Work Memory and Stockings of Cambridge.

Results: Significant difference between two groups was found on Hamilton Rating Scale for Depression ($p < 0.01$). Behavioural changes, in the form of apathy, were revealed ($p < 0.01$). Also, significant difference was found on Mini Mental State Test, as a sign of cognitive impairment ($p < 0.01$). The most striking impairments were found on tests of verbal fluency- Letter Fluency Test ($p < 0.01$), Category Fluency Test (Animal Naming Test) ($p < 0.01$), indicating executive dysfunction on Boston Naming Test ($p < 0.01$), as a sign of frontal lobe dysfunction. The differences between groups, found on tests of Spatial Work Memory (strategy score) ($p < 0.05$), and Stockings of Cambridge ((initial thinking time) ($p < 0.01$) indicate deficits in planning, attention and working memory.

Conclusion: Behavioural changes in the form of apathy and selective cognitive impairment mostly in the form of verbal fluency deficits, indicates that executive dysfunction is present in ALS patients.

P153 THE FRONTAL ASSESSMENT BATTERY IN ALS

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Keywords: Cognition, executive function, metric

Background: Many persons with ALS suffer from clinically important cognitive impairments. Executive function, a domain of higher-level cognitive processing served by the frontal lobes, is the cognitive domain most affected.

A diagnostically-sensitive and time-efficient method of identifying cognitive impairment among persons with ALS is needed. The Mini-Mental State Examination (MMSE) is the most widely-used brief assessment of cognitive function; however the MMSE contains few items on which performance reflects executive function.

The Frontal Assessment Battery (FAB) was designed as a practical assessment of executive function. Dubois et al. derived the FAB's six items from well-accepted assessments of frontal lobe function. They demonstrated that the FAB was more sensitive to executive dysfunction than the MMSE, that FAB and MMSE performance were not correlated, and that performance on the FAB varied as a function of the severity of frontal lobe disease.

The FAB is brief (5–10 minutes) and is easily learned by all clinicians. Additionally, normative data for age and education is available.

Objectives: To determine if the FAB is well suited for use as a 'bedside' screening assessment of executive function among persons with ALS, and if the FAB is a better measure of cognitive dysfunction in ALS than the MMSE.

Methods: We recruited 18 subjects who were not clinically demented for a pilot trial at the University of Colorado. Their mean ALS-FRS was 35 (range 22 to 45). All subjects underwent FAB and MMSE testing. Two patients were excluded from the analysis; one due to being unable to perform several of the tasks on the MMSE and the FAB, and one due to missing data.

Raw MMSE and raw FAB scores were Z-transformed and normalized using normative databases for age and education. A Z-score of ≤ -2 defined impairment on the MMSE and FAB.

Results: FAB score mean was 15.1 ($\pm$1.93) out of 18, and the MMSE mean was 29.0 ($\pm$ 0.894) out of 30. The FAB detected impairment in half (8/16) of the subjects as defined by FAB Z-scores ≤ -2. The MMSE did not detect any abnormalities, none had MMSE Z-scores ≤ -2. The mean FAB Z-score was $-2.14(\pm 2.26)$, which is lower than the mean MMSE Z-score of 0.108 ($\pm$0.819), $p = 0.024$. Analyses of unadjusted scores revealed the same pattern of results. FAB scores were not significantly correlated with MMSE scores.

Discussion: This preliminary experience with the FAB suggests that the test will work well as a screening test for cognitive impairment for the majority of persons with ALS. MMSE is a poor measure with which to screen for the presence of executive dysfunction. Further testing comparing the FAB to a "gold standard" battery of neuropsychological tests and other proposed bed side screening tests is warranted.

P154 THE ALS COGNITIVE BEHAVIORAL SCREEN (ALS CBS™): PHASE II VALIDATION

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Keywords: Cognition, Screening, Behavior

Background: Screening for cognitive and behavioural impairments is important in clinical settings when time and resources for detailed testing are limited, or when collecting longitudinal data to demonstrate change is needed. The ALS Cognitive Behavioral Screen (ALS-CBS™) is an assessment tool, developed in our center, containing a compilation of tasks and questions sensitive to frontal lobe dysfunction. The cognitive portion consists of 5 domains specific to frontal lobe function, while the behavioral portion consists of 15 caregiver-directed questions assessing change since disease onset. The ALS-CBS is a 5-minute screen completed in a routine clinical setting by members of the care team.

Objectives: Report current data on the ongoing validation of the revised version of the ALS-CBS™.

Methods: Previous validation resulted in several modifications to enhance accuracy. The current form of the ALS-CBS™ was administered to 40 consecutive ALS patients at two multidisciplinary centers. Patients underwent direct testing by a staff member for the cognitive score, while the behavioral portion was simultaneously completed by the caregiver. Screens were reviewed by the lead neuropsychologists for accuracy and consistency. Descriptive statistics were run to evaluate distribution of scores and correlation with disease-related variables.

Results: The average age of patients ($N = 40$) at the time of testing was 58 years and mean education was 15 years. Mean FVC ($N = 20$) was 68% and mean ALS-FRS-R ($N = 20$) was 32/48. On average, patients were tested 38 months from the time of first symptom. The mean cognitive score among all ALS patients was 15.5 (3.5) (total possible score: 20) versus 18.3 (1.0) in controls. ALS patients who met Neary Criteria for FTD had a mean cognitive score 0/20. Behaviourally, caregivers reported an average score of 33 (8) out of a total of 42 points, reflecting mild changes in behaviour in non-demented ALS patients. The ALS-FTD group had significantly lower behavioural scores (mean: 11). FVC and cognitive scores were not correlated, and there was a mild correlation ($r = 0.23$) between the cognitive score and ALSFRS-R.

Discussions and Conclusions: Our data provide evidence supporting the utility of a standardized, brief assessment tool for recognizing cognitive and behavioural impairment in the clinical setting. Continued development and validation of the ALS-CBS is currently underway. Six multidisciplinary ALS centers are involved in data collection for this measure, and additional data from these centers will be evaluated in the near future. The goal of this validation is to provide normative data to maximize interpretation of scores, including validation against the gold standard (neuropsychological test battery) and comparison to a larger cohort of normals.

P155 ASSESSMENT OF DEPRESSION IN ALS: A COMPARISON BETWEEN A PHYSICAL-RELATED AND A PSYCHOLOGICAL-RELATED SCALE

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Keywords: Depression, physical symptoms, psychological symptoms

Background: Previous studies showed that the frequency of overt depression in ALS patients is relatively low (18 to 30%). However, it is still unclear whether the scales used to assess depression in ALS includes items about physical consequences of depression as "I have crying spells or feel like it" or " I notice that I'm losing weight" that are normal consequences of ALS physical impairment. These items may seriously spoil the results of depression in ALS patients.

Aims: The aims of this study were: (a) to compare two different depression scales, one related to physical consequences of depression and one focused only on psychological aspects of depression; (b) to correlate these scales to patients physical status; and (c) to find any correlations between depression, anxiety, and quality of life.

Methods: 78 consecutive ALS patients were interviewed. Zung depression scale (ZDS), Hospital anxiety and depression scale (HADS), McGill quality of life questionnaire (MQOL) were administered. Correlations were assessed with Pearson's correlation ($p < 0.05$), comparison between means was evaluated with student t-test ($p < 0.05$). Patients' physical status was evaluated with ALS-FRS.

Results: Patients mean age was 62.1 (SD 11.8); their mean number of years of formal education were 9.5 (SD 4.5), 42 were men (53.8%). Mean anxiety (HADS) score was 7.2 (SD 7.2 range 1–14), mean depression (HADS) score was 5.6 (SD 3.4 range 1–14), mean ZDS score was 40.1 (SD 8.8 range 20–80), mean MQOL score was 7.2 (SD 1.2), mean ALS-FRS score was 28.9 (SD 8.1). Correlation between depression assessed with ZDS and HADS was significant ($p = 0.0001$); there was also a significant correlation between anxiety and

depression ($p = 0.0001$) and between quality of life and anxiety ($p = 0.000$), or depression ($p = 0.006$).

Conclusions: The significant correlation between ZDS score and HADS score suggests that, despite the presence of several items related to physical impairment in the former scale, the two scales for depression give similar results in ALS. A strong relationship between depression and anxiety was found: patients who had a strong negative reaction to the illness showed both anxiety and depression.

P156 ALEXITHYMIA IN ALS-A NEUROPSYCHOLOGICAL APPROACH

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Keywords: Alexithymia, Neuropsychology, Psychopathology

Background: Depression and anxiety in amyotrophic lateral sclerosis are not that frequent. Patients usually seem to accept the diagnosis without distress. It is of interest to study interactions between the psychology of emotions and the amyotrophic lateral sclerosis patient's cognitive processes.

Objectives: We studied the relationships between alexithymia, characterized by an incapacity to express emotions, and the cognitive perturbations observed in amyotrophic lateral sclerosis. The aim of this research was to answer two questions: 1) are amyotrophic lateral sclerosis patients alexithymic? 2) are the cognitive perturbations observed in amyotrophic lateral sclerosis responsible for alexithymia?

Methods: There were 14 ALS patients, 11 men and 3 women, aged 47 to 81 yrs, mean = 64.43 ($\pm$9.33), ALS since 45 months ($\pm$36), mean ALSFRS score 39.8/48 and 9 control subjects, 2 men and 7 woman, aged 50 to 79 yrs, mean = 61.89 ($\pm$9.1). They answered to the following questionnaires and tests: Toronto's Alexithymia scale at 20 items (TAS 20), Beck's depression scale (BDI II), Apathy scale (Robert *et al.*, 2002) and a battery of executive function (Stroop, Verbal Fluency, Trail Making Test, Empans and Clock).

Results: Reach statistical significance was observed on the Stroop Test ($p = .0024$) and verbal fluency for both naming form ($p = 0.004$) and category ($p = 0.0012$). The TAS 20 score was higher ($p = 0.0198$) in ALS patients. There was one positive correlation between depression and alexithymia ($p = 0.0214$).

Discussion and Conclusion: ALS patients are alexithymic and present slight cognitif deficits, but slight cognitif deficits and alexithymia are not correlated. ALS patients present a difficulty to stand their selective attention in inhibition tasks, a lack of research strategies in memory and a bad capacity of lexical initiation.

Therefore, it is possible to consider alexithymia in ALS patients like a defensive and adaptative process, to cope with anxiety, stress and the reality of death.

P157 CONTROLLING FOR VEGETATIVE SYMPTOMS IN THE ASSESSMENT OF DEPRESSION: IS MOOD RELATED TO DISEASE SEVERITY IN ALS?

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Keywords: Depression, Assessment, Vegetative

Background: There is substantive current research on depression in ALS but little is known about the relationship between mood symptoms (i.e. sadness, hopelessness) or the evaluative symptoms (i.e. self-criticism, guilt or shame) of depression and disease severity once vegetative symptoms are controlled for. Vegetative symptoms of depression (i.e. fatigue, sleep disturbance) may confound the diagnosis of depression in ALS patients. The Chicago-Multiscale Depression Inventory is a novel measure that has not been used in the ALS population. This measure, developed specifically for use with medical patients, may be useful given its construct of controlling for the vegetative symptoms of depression.

Objectives: To examine the relationship between the mood symptoms of depression and the severity of disease process in ALS while controlling for vegetative symptoms.

Methods: Fifteen subjects with ALS, ages 33–78, volunteered to participate in an imaging study. Subjects completed the Chicago Multi-scale Depression Inventory (CMDI) and the Beck Depression Inventory (2^{nd} ed.) (BDI-II) as part of a larger neuropsychological battery. The CMDI provides standardized scores for subscales of mood, evaluative, and vegetative symptoms, as well as a total score. Patients received neurological examinations including forced vital capacity testing (FVC) and the ALS Functional Rating Scale-revised (ALSFRS-R). Correlations were completed using Pearson's r correlation analyses.

Results: The prevalence of clinically significant mood symptoms of depression was 27%, as measured by either the Mood subscale or the Evaluative subscale of the CMDI. A strong correlation existed between the CMDI Mood subscale and disease severity as measured by the ALSFRS-R ($r = -0.55$) and FVC ($r = -0.45$). Mood symptoms were weakly correlated with disease duration ($r = 0.168$). The Vegetative subscale of the CMDI was not significantly correlated with FVC or ALSFRS-R and was modestly correlated with duration of symptoms ($r = -0.37$). The Evaluative subscale of the CMDI was highly correlated with ALSFRS-R ($r = -0.58$), and FVC ($r = -0.75$) but weakly correlated with symptom duration ($r = 0.26$) and the Vegetative subscale ($r = 0.29$).

Discussion and Conclusions: These results suggest that the mood and evaluative symptoms of depression in this sample were associated with severity of disease process as measured by ALSFRS-R and FVC, but not related to disease duration. The finding that the vegetative symptoms of depression were weakly correlated with disease process might suggest denial or lack of insight into motor-related physical disability. The results of this study suggest the need to focus on evaluative symptoms of depression in relation to disease progression in future research.

P158 A STUDY ON COGNITIVE FUNCTION IN MOTOR NEURON DISEASE

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Keywords: cognitive impairment, frototemporal dementia, neuropsychiatry inventory

Objectives: To investigate the frequency of cognitive impairment and frontotemporal dysfunction in motor neuron disease (MND).

Methods: One hundred cases of MND patients during December 2006 to October 2007, underwent mini mental state examination (MMSE) for cognitive survey and neuropsychiatry inventory (NPI) for neuropsychological survey, meanwhile Hamilton depression scale (HAMD) and Hamilton anxiety scale (HAMA) for mood statement. Demographics, site of onset and ALS-FRS were also investigated.

Results: Of the 100 patients (60 male and 40 female, age range from 24 to 78 years, mean 51.99 ± 11.41 years), 45 were diagnosed as definite ALS, 29 as probable ALS, 16 as possible ALS, and 10 as lower motor neuron syndrome (LMNS). Eighty-two had limb onset and 18 bulbar onset. Sixty-one patients and bulbar dysfunction. The course of disease was 4–94 months, confirmed diagnosis for 0–47 months. Only depression state and ALS-FRS were of statistical difference between MMSE normal and abnormal. MMSE indicated that 24.2% (23/95) of all patients were mild cognitive impairment. 15.8% (15/95) of all patients had abnormal in both MMSE and NPI. Depression presented most often in all the NPI subitems. Apathy, agitation, euphoria, aberrant motor behavior were proved to be of great clinical value to frototemporal dysfunction in the present study, 10 patients (10%) presented at least 1 of the 4 subitems. Since patients with anxiety and depression would also act as abnormal in NPI, we took a follow up after 3 months with anti-depression therapy. Two patients did not improve. Their psychomotor dysfunction initially emerged and lasted for more than 6 months, indicating that they may have frontotemporal dysfunction.

Conclusions: MND patients' cognitive function were relatively reserved. 1/4 of the 100 patients have mild cognitive impairment. A part of the patients were psychomotor abnormal, in the follow-up, 2 patients may have frontotemporal dysfunction, with potential to become frototemporal dementia.

P159 A POPULATION BASED SURVEY OF COGNITIVE DECLINE IN ALS

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Keywords: cognitive, FTLD, population

Background: Up to 60% of the ALS patients attending specialist clinics are reported to have mild cognitive decline and a proportion of these may progress to develop frontotemporal dementia. However, the population-based frequency and natural history of cognitive decline in ALS remains to be established.

Objective: To determine the frequency and natural history of cognitive decline in ALS in a defined population-based cohort.

Design and Methods: All Incident patients in the Republic of Ireland are identified through the Irish ALS Register. Each patient is assessed in their own home, provides a detailed family history and undergoes a full neurological examination and validated neuropsychological evaluation, modified to control for slower motor speed and speech difficulties. Progression is determined by three separate evaluations at 6-month intervals.

Results: 70 incident patients have been identified over an 18 month period. 51 patients and matched controls have participated in a detailed neuropsychological assessment to date. 13 patients have bulbar-onset disease and 17 have a family history of neurodegenerative disease. Cluster analysis based on executive function was performed to generate 2 groups: impairment (n = 23) and no impairment (n = 28). ALS patients demonstrated performance below control means on several measures of executive function and memory. Patient mean z-scores indicate verbal memory and behaviour change were more prominent in those with evidence of executive impairment. Those with executive impairment had significantly greater change in executive dysfunction and apathy (as per Frontal Systems Behaviour Scale). Apathy was the most marked behavioural change in both groups. Other cognitive processes such as naming were intact.

6 patients met consensus criteria for frontotemporal lobar dementia: 3 for behavioural variant FTD, 1 for non-fluent progressive aphasia, and another for semantic dementia. Cognitive impairment did not correlate with ALS severity. Kindreds of ALS patients with cognitive impairment had a higher frequency of other neurodegenerative disease.

Conclusion and Relevance: There is a high prevalence of cognitive impairment (primarily affecting executive function and memory) and behavioural change in ALS. However preliminary data suggests that the population based frequency of cognitive impairment is lower than that reported from clinic based studies. This study supports the conjecture that ALS is part of a continuum of neurodegenerative disease.

P160 PREVALENCE AND SEVERITY OF DEPRESSION IN MOTOR NEURONE DISEASE (MND) AND OTHER MOTOR DISORDERS

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Keywords: Severity, Prevalence, Depression

Background: The prevalence of depression in Motor Neurone Disease (MND) is portrayed in the research literature as being surprisingly low. This impression has been reinforced by reports that the prevalence of depression is lower in MND than in several other neurological conditions. The disorders (such as Parkinson's disease, Multiple Sclerosis) to which prevalence rates of depression in MND have been compared are characterised by additional neuroanatomical, cognitive and behavioural features that have been reported to exacerbate depression. These additional confounds render comparisons of prevalence rates of depression between MND and these conditions less helpful and the conclusions drawn from such comparisons in need of review.

Objective: The current study sought to examine whether the prevalence in depression is uniquely low by comparing the severity and prevalence of depression in patients with MND to

that in patients with other neurological motor disorders that have a similar physical disability profile to MND.

Method: The Beck Depression Inventory (revised) (BDI-II), the Major Depression Inventory (MDI), Hospital Depression and Anxiety Scale (HADS) and the Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised were sent to 225 patients from a tertiary referral Motor Nerve Clinic in London, UK.

Results: Data were obtained collected from 109 patients: 63 with MND and 46 with other neurological motor disorders. After covarying for potentially confounding demographic and functional ability variables, ANCOVAs revealed no significant effect of patient group on total scores on the BDI-II, MDI or the HADS depression subscale. The prevalence of depression did not differ significantly between patient groups according to classifications from the BDI-II, HADS depression subscale and the MDI.

Discussions and Conclusions: The current findings contradict the impression that patients with MND have a surprisingly low prevalence of depression. Patients with similar disability profiles to MND did not significantly differ from MND patients with regard to both severity of depressive symptoms and prevalence of depression on the BDI-II, HADS or the MDI. The conclusions have significant implications for our understanding of the psychological impact of MND and suggest that the impression of a generally low prevalence of depression in MND patients is revised. Given the negative impact of depression on quality of life and the potential impact of mood on both patients' acceptance of life-prolonging measures and prognosis, the possibility of depression in people with MND should be given due consideration.

P161 ALS PATIENTS WANT TO BE TOLD MORE ABOUT COGNITIVE CHANGE

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Keywords: Cognition, information seeking, neuropsychology

Background: Once thought to impact only voluntary motor function, ALS/MND is now seen as a multi-system disorder in which a minority of patients experience mild cognitive dysfunction or frontotemporal dementia. Despite clinical guidelines advocating supplying complete information to patients, educational materials on ALS often state that the mind is unaffected.

Objectives: We sought to establish how much patients and caregivers understand about ALS, what they have been told to expect by their physician, and if they would have appreciated more complete information.

Methods: A two-part survey was administered online. An "ALS quiz" gauged participants' knowledge of physical and psychological aspects of ALS. A second questionnaire assessed which symptoms patients had discussed with their clinician and explored patients' desire to receive information on psychological effects.

Results: 247 ALS patients and 87 caregivers participated. Participants knew less about psychological symptoms than physical ones (72% correct responses versus 82%; paired $t_{(333)} = -5.04$, $p < 0.001$). Patients commonly reported being told by their doctor about physical symptoms such as problems walking (85%) or stiffness/cramps (74%) but not psychological issues like emotional lability (46%) or cognitive change (11%). The majority of patients (62%) and carers (71%) indicated a desire to be informed that cognitive change or dementia might occur.

Discussion: Although we as healthcare professionals know that cognitive change can and does occur in ALS, we are not telling patients. It is incumbent upon as a profession to find the best way and the most appropriate time to tell patients what could happen, so that if cognition does become an issue, they are prepared for it. We could learn from the experiences of colleagues in Parkinson's disease and Multiple Sclerosis for guidance.

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THEME 9 RESPIRATORY AND NUTRITIONAL MANAGEMENT

P162 DOES TASTE PERCEPTION CHANGE WITH ALS?

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Keywords: ALS, taste, MND

Background: The traditional concept that patients with ALS have disease limited to the motor system has been challenged in recent years. It is well known that ALS patients frequently lose weight, attributed to muscle atrophy as well as dysphagia. However, some subjects also report less interest in food and/or taste changes. It is also known that focal taste damage may not be perceived with whole mouth tasting (as in eating) due to disinhibition of the taste cranial nerves(1), and that aging and gender affect taste perception(2,3).

Objective: It was hypothesized that focal taste damage may occur in ALS patients compared to healthy controls.

Methods: Two age-gender matched adult groups were recruited (n = 22; 11 ALS and 11 healthy adults). All ALS patients received a clinical swallowing examination prior to participation. The Spatial Taste Test was administered using 1.0M NaCL, 1.0M sucrose, 0.032M citric acid, 0.001M quinine hydrochloride diluted in deionized water (4). Taste identification, whole mouth and bilateral taste intensity ratings on the fungiform and circumvallate papillae were conducted using the generalized Labeled Magnitude Scale, which has been shown to have validity across groups (5). Independent 2-sample t-test analyses were performed between groups for all tastes, laterality and papillae including whole mouth taste intensity. P-values ≤ 0.05 were considered significant.

Results: Compared to healthy age/gender matched volunteers, ALS patients exhibited significantly lower intensities for sweet and sour tastes in the fungiform papillae bilaterally. No significant differences were observed in the circumvallate papillate or whole mouth taste intensities. A trend of lower intensities across all tastes and papillae was observed in ALS patients vs healthy volunteers. Five of the 11 ALS patients reported taste changes; 3/5 had tongue fasciculations.

Discussion: Results of this small pilot study suggest ALS may alter taste perception with focal damage to the chorda tympani which innervates the fungiform papillae and may include the glossopharyngeal which innervates the circumvallate papillae. Although the hypothesis was confirmed, caution must be exercised when interpreting the results due to the small sample size. The mechanism for taste loss or changes with ALS is unknown. It is not known whether abnormal movement of a taste bud due to fasciculations will alter taste perception.

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P163 A CARE PATHWAY FOR PEOPLE WITH MOTOR NEURONE DISEASE NEEDING NUTRITIONAL SUPPORT

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Keywords: nutrition, pathway, gastrostomies

Background: It is widely accepted that for patients with Motor Neurone Disease (MND) with bulbar symptoms, the early siting of an enteral feeding (EF) tube i.e. PEG (percutaneous endoscopic gastrostomy) or RIG (radiologically inserted gastrostomy) optimises care compared to later placement when a patient may be malnourished, dehydrated, or have respiratory insufficiency.

As part of a project to improve the care of people with MND, a multi-disciplinary team (MDT) of professionals examined the care of people needing nutritional support (NS): particularly when and how to broach the subject of NS with the patient; decision making over PEG vs RIG vs NGT (nasogastric tube) placement; and streamlining the process.

Objectives: To devise a care pathway to expedite the processes of referral and assessment for the appropriate EF tube. To improve the quality of life for patients with bulbar symptoms.

Methods: A task and finish group of MDT members (dietitians, nurse specialists in MND, doctors in palliative care, respiratory medicine, gastroenterology and radiology) examined current practice, and a literature search about best practice informed the new pathway. It was then refined as patients used it. Training needs were identified to enhance the assessment of respiratory function pre-procedure and facilitation of NG tube placement in the community. Documentation of timing and type of tube placement, use of tube (i.e. initially for hydration or immediately for total nutritional support) and the post-placement morbidity was recorded on a database. Patients and carers were asked about their experiences.

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Results: An evidence-based care pathway has been developed. Prior to this pathway, PEGs were the only EF tube offered. The pathway has allowed the appropriate use of RIG and NG tubes. Measurement of sitting and lying forced vital capacity has provided a more objective assessment to inform decision making on the most appropriate EF tube.

Discussion and Conclusions: This care pathway has drawn together best practice for patients with MND needing nutritional support. We have discussed NS with patients earlier, so that timely, informed decisions can be made, with verbal feedback from patients and carers that this has empowered them and improved quality of life. A DVD resource is being developed to collate patient/carer comments so future patients can gain further insights. Training for the Home Enteral Nutrition Dietitian on NG tube placement for either short-term hydration purposes prior to PEG/RIG placement or to prevent acute hospital admissions at end of life has further improved overall choice. RIG placements are now appropriately offered and the process by which any EF tube is placed has been expedited. This initiative is already being examined by other specialist groups within our trust as an example of good practice.

P164 RESTING ENERGY EXPENDITURE (REE) IN PATIENTS WITH ALS

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Keywords: metabolism, calorimetry, nutrition

Background: Nutrition is a prognostic factor for survival in patients with ALS. Known factors that contribute to poor nutrition include dysphagia, anorexia, fatigue, and constipation. In addition, research has shown that patients with ALS exhibit an increase in metabolism (1). Sherman, et al. showed that standard equations are not accurate in determining REE in patients with ALS (2); therefore, more accurate caloric needs should improve patient care. We propose that the best method to determine energy expenditure in the ALS population is indirect calorimetry.

Objectives: i) To understand better the metabolic requirements and nutritional needs of patients with ALS. ii) To Demonstrate the robustness of indirect calorimetry in ALS patients in an outpatient setting. iii)To Compare REE to gender, age, height/weight/BMI, FFM, FVC, VO2, diet status, ambulatory status, and disease onset.

Methods: 77 patients (43 male/34 females) with ALS (56 spinal/21 bulbar onset) were measured for REE using indirect calorimetry (KORR MetaCheck Metabolic Analyzer Model 7100.) Standard procedures were followed for the MetaCheck, except we used a face mask rather than the provided mouthpiece to ensure adequate seal.

Results, Discussions and Conclusions: 72 of the 77 patients completed the MetaCheck analysis. Five were excluded on account of low tidal volumes. REE was found to be higher in the ambulatory and oral feeding patients as compared to the wheelchair-bound and tube-fed patients. REE decreased as age increased. Patients with lower FVC levels had a lower REE compared to higher FVC levels. Bulbar patients had a lower REE than spinal patients. Indirect calorimetry successfully estimated REE. Different patient populations (e.g. spinal vs. bulbar) require different calorie intake to maintain nutrition.

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P165 INDIRECT CALORIMETRY IN THE OUTPATIENT ALS SETTING

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Keywords: metabolism, indirect calorimetry

Background: Research has shown that patients with ALS exhibit an increase in metabolism (1). Sherman, et al. showed that standard equations are not accurate in assessing REE in patients with ALS (2). Therefore, determining accurate caloric needs should improve the standard of patient care.

Indirect calorimetry is considered the only accurate and clinically feasible method of measuring energy expenditure. We propose that the best method to determine energy expenditure is indirect calorimetry and can be a routine test in the outpatient ALS clinic.

Objectives: Demonstrate the use of indirect calorimetry in ALS patients in an outpatient setting. Obtain accurate results with good compliance to the test procedures. Determine whether indirect calorimetry is cost effective, patient friendly, as well as easy for staff to use in the outpatient clinic.

Methods: 96 patients (53 male/43 females) with ALS were measured for REE using indirect calorimetry (KORR MetaCheck Metabolic Analyzer Model 7100.)

All patients were instructed to fast for 4 hours, avoid all caffeinated beverages, and avoid exercise on the day of the Metacheck. Patients were seated at rest for 15 minutes prior to the procedure. The test was adapted to use a face mask rather than the provided mouthpiece to ensure an adequate mouth seal. The testing time is 10 minutes of breathing at a normal rate through the face mask.

Results, Discussions and Conclusions: Goal of the clinic is to obtain a Metacheck for every patient. This would require all patients to fast for 4 hours prior to their clinic visit. Initial clinics proved this to be too difficult due to the patient load and prolonged fasting period. Standard procedures are being developed for easier patient compliance and improved patient comfort. This would include scheduled Metacheck times for pre-determined patients at different intervals during clinic. Team members were surveyed in the multidisciplinary clinic on the use of the Metacheck. Feedback revealed that the Metacheck did not impair others from seeing patients or prolong their visit time. The Metacheck was determined to be easy to use, required no special training for operation, and cost effective. Outcomes showed good compliance with the simple 10 minute test and the minimum 4 hour fasting period. 90 of the 96 patients were able to complete the MetaCheck analysis. Tidal volumes were too low to register in 5 patients and 1 patient was too uncomfortable to complete the test.

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2. Sherman MS *et al.* Journal of Parenteral & Enteral Nutrition 2004; 28(6):442–6.

P166 MODELING ENERGY EXPENDITURE IN ALS PATIENTS

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Background: A clinical trial of 80 ALS patients underwent a protocol using doubly labelled water (DLW) to determine total daily energy expenditure (TDEE). DLW measurements were taken at days 1, 7 and 10. In addition, on each of these days measurements of fat free mass were obtained by both DEXA and BIS. On days 7–10, detailed dietary information was taken in addition to measures of physical activity determined by Actical accelerometers.

Objective: Determine how well established TDEE formulas predict actual TDEE values obtained from DLW measurements in ALS patients and to assess how to improve the predictive ability of such formulas by the inclusion of patient demographics, fat free mass, forced vital capacity measurements, ALS-FRS baseline score, and ambulatory status predict TDEE.

Methods: Multiple regression models were used to make the predictions. Various models based on the Harris Benedict equation the Wang equation the Columbia equation, and the Vermont equation were used as starting points. Models were supplemented by adding in patient demographics, forced vital capacity measurements (erect and supine), ALS-FRS score, and ambulatory status.

Results: These patients are 35% female, have a mean age of 59 ± 11.7 years, and a mean of 14.26 ± 2.88 years of education. At baseline, these patients had a mean ALS-FRS of 36.1 ± 5.82 and mean%FVC of 76.13 ± 15.85. The mean time from disease onset to entry in the trial was 23.8 ± 19.67 months, and 69.6% had some ambulatory deficiency. Baseline TDEE measurements ranged from 1199 to 3614 kcal/day with a mean of 2276 ± 600. For the 63 participants with available DLW results, all of the above prediction equations underestimate the true energy expenditure by 689, 957 calories/day at baseline.

Discussion: TDEE as measured by DLW is substantially higher than the values predicted by the established formulas, and suggests that ALS patients may expend many more calories than standard methods of estimation would indicate. Since maintaining the appropriate energy balance is important for ALS patients, models that accurately predict TDEE need to be developed for this special population.

P167 DIETARY INTAKE OF PATIENTS WITH MOTOR NEURONE DISEASE/AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Keywords: dietary,fibers,fats

Introduction: Motor Neuron Disease (ALS) represents a group of neurodegenerative disorders caused by impairment of motor neurons, leading to progressive muscle weakness and muscle atrophy. Respiratory failure, dysphagia and body composition alterations are present in patients, especially in those with bulbar involvement.

Objective: The purpose of this study is to analyze the dietary intake of patients with MND/ALS.

Method: The diet of 44 ALS patients was analyzed, from May/07 to November/07 through ALS patients recalling their food intake over a 24 hour period to the nutritional services. Support to the Nutrition Data System version 2.5a- DIS-UNIFESP/EPM software, was used in this study. A nutritional classification was performed by the body mass index (BMI) by WHO (1989). For patients who had a BMI below 20.9 m^2/kg, the adjustment for women was to 21kg m^2/kg and for men, 22 kgm^2/kg. For the patients that had BMI above 25 m^2/kg, this value was adjusted to 24.9 m^2/kg. The basal metabolism was calculate by Harris Benedict equation (1919) and was accreted by the factor of 1.4, to find the total energy necessary. The dietary fiber was adjusted by using the FDA (Food Drugs Administration). For the fat percentage, the range of 25–30% of total caloric value and the relationship of 1:1 saturated fat and unsaturated was used. The reference to the percentage of saturated fat is 10% of lipids totals. The reference standard for water intake (expected) was the consumption of 35 ml/kg/day. The classification of the intestinal function was normal or constipated.

Results and Discussion: The average of patients' age was 57 years old; there were 23 male and 21 female patients. As for the nutritional status 8 patients (18.18%) showed under nutrition, 3 patients (6.81%) severe degree III and 5 (11.36%) degree I, 25 (56.81%) had atrophy in 11 (25%) showed excess weight, and 10 (22.72%) with overweight and 1 (2.27%) as Obesity degree I. Patients of both sexes reporting the caloric intake values of 1.888 Kcal, when the recommended value was 1.954 Kcal. The average fiber rate in the diet, for both sexes, was 12 grams in 1888 calories below the recommended; 62% of the patients presented with constipation. The fat total value present in the diet, for both sexes, exceeded the upper limit of 30%, presenting 36%. The saturated fats proportion in the diet had the average of 1:1,8 above the benchmark. The percentage of saturated fats on the total lipids in the diet, for males was, on average, by 10% and for females was 24%. As for the liquid intake there was an average of 911 ml, and the recommended average is 2131 ml, showing the patients consumed only 43% of the necessary.

Conclusion: The dietary consumption by patients with MND/ALS showed that the energy consumed in the average is below needs, is low in fiber, high in fat and low liquid consumption, resulting in constipation and conditions favourable to inflammatory processes by the high intake of saturated fats. Patients are atrophic, in the majority, with similar parts of undernourished and overweight.

P168 CORRELATION AMONG BODY MASS INDEX AND SURVIVAL AFTER PEG INSERTION IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Keywords: BMI, PEG, survival

Background: Patients suffering from amyotrophic lateral sclerosis (ALS) often have to face progressive nutritional problems leading at last to percutaneous endoscopic

gastrostomy (PEG) placement. The body mass index (BMI), or Quetelet index, is a statistical measure of person's weight scaled according to height: BMI is defined as the individual's body weight divided by the square of height.

Methods: We evaluated 14 spinal-onset or bulbar-onset ALS patients (5 men and 9 women; mean age ± SD: 63 ± 11 yrs) with PEG. Many data (BMI, phase angle and other soft tissue analysis parameters) were collected just before PEG insertion and correlated with survival after PEG insertion. Patients were divided into 3 groups: A group with BMI ≤ 18.5; B group with BMI between 18.5 and 25; and C group with BMI ≥ 25.

Results: By comparing A (mean survival (days) ± SD: 197 ± 272; mean resistance ± SD: 816 ± 154; mean reactance ± SD: 58 ± 14; mean phase angle ± SD: 4.3 ± 1.8) with B (mean survival (days) ± SD: 370 ± 294; mean resistance ± SD: 656 ± 115; mean reactance ± SD: 54 ± 11; mean phase angle ± SD: 4.9 ± 1.1), or C group (mean survival (days) ± SD: 392 ± 248; mean resistance ± SD: 545 ± 60; mean reactance ± SD: 55 ± 2; mean phase angle ± SD: 5.9 ± 0.9), we found significantly ($p < 0.05$) enhanced survival values in B and C groups as compared to A group; whereas B and C groups did not significantly differ from each other.

Discussion and Conclusions: Our results support the concept that early PEG placement could enhance survival in ALS patients.

P169 PERCUTANEUS ENDOSCOPIC GASTROSTOMY IN PATIENTS WITH MOTOR NEURONE DISEASE/AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: Percutaneus Endoscopic Gastrostomy, nutritional status, survival

Background: Amyotrophic Lateral Sclerosis (ALS) is a degenerative, progressive and irreversible neurological disease, that affects motor neurons. Among the symptoms, we have reduction of forced vital capacity (FVC), dysphagia, weight loss and compromised nutritional status. Nutritional and respiratory support is the most important therapeutic interventions, with use of BiPAP and Percutaneus Endoscopic Gastrostomy (PEG) (a technique developed in 1980 as an alternative to surgical gastrostomy). It is suggested in patients with high surgical risk, and it can be done in outpatient department, under sedation and with decrease of morbility and absence of complications in more than 95% of cases.

Objective: To describe the evolution of nutritional status after PEG and its relation with survival.

Methods: From February/2000 to May/2007, 111 patients with ALS by El Escorial modified criteria were enrolled in a prospective non-contestant study. PEG was performed in these patients when a modified criterion from the proposed ones by Silani in 2000 was observed. Sedatives were midazolam 0.035 to 0.07mg/kg and fentanil 0.05 to 0.1 mg/kg. We evaluated nutritional status by body mass index (BMI) before and after 30 days of PEG insertion. FVC was measured before PEG insertion and BiPAP was used by Kleopa et al criteria.

Results: Mean age was 58.3 years (11.7); there were 39 males (66.1%) and 20 females (33.9%) with ALS and 18 males (34.6%) and 34 females (65.4%) with bulbar form. Before PEG insertion, classification of patients' nutritional status was: 51.3% atrophy, 22.5% overweight and 26.2% malnutrition. FVC at the moment of PEG insertion varied between 13% and 86%, with mean of 41% (11.91). Good BiPAP tolerance was observed in 42.6% of patients, poor BiPAP tolerance in 31.7% and patients without BiPAP despite necessity were 16,8%. After PEG insertion nutritional status was: 49.5% was atrophy, 20.7% overweight and 29.7% malnutrition. Patients with bulbar form had high predominance of disturbance. Mean survival in ALS was 2190 days from the beginning of the first symptoms (IC 95%: 1226,0–3154,0), and in the bulbar form it was 1260 days (IC 95%: 904.5–1615.5) ($p = 0.009$, for the Long Rank Test). Death occurs usually by respiratory failure. Three patients died in the first 30 days after PEG insertion, all with FVC from 47% to 54%. Though the PEG insertion in some cases with FVC below the recommended one, in these patients the mortality was low in the first 30 days.

Conclusions: 1) Mortality probably is not related to PEG insertion; 2) PEG can be used even in patients with serious respiratory failure; 3) PEG allows improvement of nutritional status; 4) Despite nutritional support, patients with bulbar form have less survival, perhaps by higher level of respiratory failure and aspirative pneumonia.

P170 NON-INVASIVE MEASUREMENT OF MOUTH TO COLON TRANSIT TIME IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Keywords: Amyotrophic Lateral Sclerosis, Autonomic, Gastrointestinal motility

Background: Amyotrophic Lateral Sclerosis (ALS) is generally considered a motor system disease. However, recent evidence exists for autonomic involvement outside of the motor system including cardiac, sudomotor and gastrointestinal (GI). While there have been studies demonstrating colonic involvement, small bowel motility has not been investigated (1). GI complications are especially important given the frequent complaints of constipation and abdominal distention, the use of medications with anticholinergic side-effects, and the management of nutritional needs through the use of tube feedings.

Objective: To examine autonomic involvement of the small bowel in ALS by quantifying gastric motility in patients with ALS compared to healthy controls.

Methods: GI motility of the small bowel was evaluated using the mouth to colon transit time (MCTT) or gastric to colon transit time (GCTT) measured via the noninvasive hydrogen breath technique (2). Seven healthy controls and six patients diagnosed with probable or definite ALS were examined. Patients were instructed to hold their medicines from the previous night. After an 8 hour fast, the baseline hydrogen content of their expired air was measured. This was followed by the administration of a measured caloric load containing 20 grams of lactulose. Hydrogen breath measurements were taken every ten minutes until there were two consecutive increases in hydrogen gas concentration, indicating the bolus had reached the colon. The time of the second increase in

hydrogen gas concentration was recorded as the MCTT or GCTT.

Results: The mean MCTT/GCTT in individuals with ALS was significantly prolonged compared to controls (121 vs. 74 minutes, $p = 0.002$). MCTT/GCTT increased as ALS-FRS-R scores decreased ($R = -0.72$) indicating progressing small bowel involvement as the disease worsens.

Conclusions: These results indicate autonomic involvement of the small bowel that may increase as the disease progresses, likely contributing to some of the symptoms observed in patients with ALS. This has importance in the management of tube feedings and the use of medications that can further alter GI motility.

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P171 UNSEDATED PERCUTANEOUS ENDSCOPIC GASTROSTOMY USING AN ULTRATHIN TRANSNASAL ENDOSCOPE FOR AMYOTROPHIC LATERAL SCLEROSIS WITH RESPIRATORY FAILURE

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Keywords: PEG, transnasal endoscope, respiratory failure

Background: In amyotrophic lateral sclerosis (ALS) with dysphagia, percutaneous endoscopic gastrostomy (PEG) provides a survival benefit, and improves quality of life. However, due to the potential hazard of PEG for ALS with respiratory muscle weakness, PEG is recommended when the percent predicted forced vital capacity (%FVC) exceeds 50%. On the other hand, many patients who require PEG have already lowered respiratory muscle strength with FVC below 50%. A safe PEG method that can be used in patients with respiratory failure is required.

Objectives: Conventionally PEG is performed by the Pull method using a perioral endoscope. The Direct method using an ultrathin transnasal endoscope is less invasive and has no need for conscious sedation. We evaluated the safety and usefulness of this method in ALS patients with dysphagia.

Methods: Five ALS patients with dysphagia were studied. The nasal route was prepared before the procedure by applying a topical vasoconstrictor (naphazoline nitrate) and local anaesthetic (lidocaine hydrochloride). The GIF-XP260N endoscope (Olympus Optical Co.), which had a distal-end diameter of 5.0 mm, was inserted transnasally without conscious sedation. After air insufflation into the stomach, two-point fixation was conducted using the Funada's gastropexy device (Create Medic Co.). A button type catheter was inserted by the Direct method using a Kangaroo Seldinger PEG kit (Nippon Sherwood Medical Industries Co.).

Results: All 5 procedures were successful. The average duration of the procedure was approximately 15 min. The procedures were well tolerated by all the patients, with none requiring any sedation. There was no major complication, with no lowering of oxygen saturation and no abnormal increase of pulse rate. There was little pain associated with transnasal endoscope insertion and conversation was possible during the procedure. No peristomal infection was observed and sutures were removed after 1 week. In a patient who was receiving non-invasive ventilation (NIV), the procedure was conducted safely with simultaneous NIV support using a nasal mask with a channel created for the endoscope.

Discussion: The Direct method applying the Seldinger technique is an improved Introducer method. With the Direct method, the role of the endoscope is only for insufflations of air into the stomach, and observation of the procedure. There is no need for peroral insertion of a conventional endoscope. Recently developed ultrathin transnasal endoscopes are very fine, with external diameter of 5.0 mm. Therefore, conscious sedation is not required during the procedure. This point is very important for PEG in ALS patients with respiratory failure. Furthermore pharyngeal anesthesia is not needed, and the risk of aspiration of saliva or gastric fluid is reduced.

Conclusions: The Direct method using an ultrathin transnasal endoscope without sedation is less invasive and less painful than the Pull method using a peroral endoscope. We recommend using this safe method in PEG for ALS patients with respiratory failure.

P172 APPLICATION OF NON-INVASIVE VENTILATION DURING PERCUTANEOUS RADIOLOGIC GASTROSTOMY IN ALS PATIENTS WITH LOW VITAL CAPACITY

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Keywords: PRG, NIPPV, Nutrition

Background: Nutritional aspect in ALS patients is important in their survival and quality of lives. Percutaneous endoscopic gastrostomy (PEG) is recommended when forced vital capacity (FVC) in seated erect position is 50% of predicted value or greater. In general, PEG is a relatively safe procedure, but the morbidity and mortality rise as the FVC falls. When performing PEG, mild sedation is needed, which might be a hazard of respiratory compromise in patients with a FVC $<50\%$ and there is a risk of aspiration as the pharynx is transiently anesthetised. Some studies reported successful PEG placement in ALS patients whose predicted FVC $<50\%$ using noninvasive positive pressure ventilation (NIPPV) support by nasal mask during PEG procedure. But, respiratory compromise, insufficient ventilation and secretion care during procedure are unsolved problems. Percutaneous radiologic gastrostomy (PRG) is a good alternative method as it does not need sedation and an endoscopic tube, but, performing PRG to the patients with very low FCV is also a great burden to interventional radiologists.

Objectives: We describe the concomitant use of NIPPV during PRG placement in ALS patients with low FVC and discuss the safety and feasibility.

Methods: Twenty-five ALS patients (13 men, 12 women; mean age 55.5 years; range; 40–73 years) who had dysphagia and using NIPPV were recruited. Their pulmonary function tests were performed. During the PRG procedure, the

patients used their NIPPV via a nasal mask. For premedication, no sedatives and narcotics were used. Under fluoroscopic guidance, PRG tube placement was performed by an interventional radiologist using gastropexy with 1 ~2 T-fastener.

Results: The mean FVC in intermittent NIPPV using group (n = 16) was 1408.8 ± 694.3 ml (35.9 ± 17.7% of predicted value) in sitting position, and 1074.4 ± 469.2 ml (27.3 ± 11.1%) in supine. The mean PCF was 175.7 ± 37.3 L/min. The FVC and PCF could not be evaluated in 24 hours NIPPV using group (n = 9) because the patients rejected to be weaned from their ventilators. The PRG placement showed 100% success rate technically. There were no major complication such as aspiration, panperitonitis, and respiratory arrest. However, several minor complications happened: pnemoperitoneum (4%), peri-gastrostomy tube leakage (8%), skin irritation and suture breakage (36%), and would infection (8%). Mean survival of 25 ALS patients was 32.1 months (Keplan-Meier method, SE, 5.0, median 20.0 months). There was no difference of survival between intermittent NIPPV using group and 24 hour NIPPV using group (log-rank test, p = 0.82).

Discussion and Conclusions: Application NIPPV during PRG is successful and safe method for nutritional care of ALS Patients with low FVC.

P173 AN INVESTIGATION FOR THE USE OF NON-INVASIVE POSITIVE PRESSURE VENTILATION AND PERCUTANEOUS ENDOSCOPIC GASTROSTOMY IN CHINESE ALS PATIENTS

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Keywords: NIPPV, PEG, respiratory & nutritional managements

Backgrounds: In China, a few patients with amyotrophic lateral sclerosis (ALS) can get effective respiratory and nutritional managements in their whole disease course.

Objectives: To investigate the use of non-invasive positive pressure ventilation (NIPPV) and percutaneous endoscopic gastrostomy (PEG) in Chinese ALS patients, and analyze the potential influential factors of their current status.

Methods: Total 472 Chinese patients with ALS diagnosed from 01-01-2005 to 31-12-2007 were investigated using a specially designed questionnaire which contained 24 issues pertaining to information about the use of NIPPV and PEG.

Results: The overall percentage of patients presently using NIPPV and PEG was 16.3% and 4.4%, respectively. The percentages of use of NIPPV and PEG year by year were 3.7% and 1.9% in 2005, 10.0% and 1.7% in 2006, and 21.1% and 6.0% in 2007, respectively. Among the patients who received NIPPV therapy, the time from symptom onset to beginning of use was 0–1 years (20/77, 26.0%), 1–2 years (26/77, 33.8%), 2–3 years (14/77, 18.2%), and beyond 3 years (17/77, 22.0%), respectively. 76.2% of the patients who received PEG were operated in 3 years. The main reasons impelling ALS patients to use NIPPV included doctors' advice (96.1%), propagating brochures (67.5%), communication among patients (31.2%), persisting dyspnea (30.0%) and education meeting (15.6%). In contrast, the main causes obstructing the patients to choice NIPPV were lack of doctors' advice (47.3%), poor income (45.8%) and incomprehension to NIPPV (45.3%). Also, the main factors that made patients receive PEG were related to doctors' advice (90.5%), dysphagia (71.4%) and propagating brochures (47.6%). The key points to obstruct patients from PEG were absence of doctors' advices (50.6%), incomprehension to PEG (39.5%), dread of operation (39.5%), and thought of the inconvenience caused post-PEG (39.2%).

Conclusions: The percentages of use of NIPPV and PEG in ALS patients in China were much lower than those of patients with ALS in developed countries, but the proportion has been gradually promoting in recent years. The main influential factors to extend NIPPV and PEG in Chinese patients were doctors' advice and patient education. The restrictive ones include absence of doctors' advice, lack of knowledge about NIPPV and PEG, and lower level of income.

P174 INDICATORS OF EARLY RESPIRATORY FAILURE IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: SNIF, diaphragmatic compound muscle action potential, NIV

Background: It has been shown that non-invasive positive pressure ventilation (NIV) may be effective in improving prognosis and maintaining QOL in patients with amyotrophic lateral sclerosis (ALS). However, it is difficult to determine the optimal timing of NIV initiation because the modes of onset and progression vary widely in ALS.

Objectives: The aims of this study were: (1) to elucidate a possible role of sniff nasal-inspiratory force (SNIF), diaphragmatic compound muscle action potential (DCMAP) and conventional pulmonary function parameters as indicators of early respiratory failure in patients with ALS; (2) to establish an appropriate protocol to predict the optimal timing of NIV initiation.

Methods: 30 patients who were diagnosed as probable ALS (El Escorial criteria) were subdivided into 4 groups according to the site of onset as follows: group A; upper limb onset, group B; lower limb onset, group C; bulbar onset, and group D; respiratory muscle onset. Diaphragmatic functions were evaluated using SNIF measurements and DCMAP recordings by electrical phrenic nerve stimulation at the neck. Blood gas analysis, forced vital capacity (%FVC), DCMAP, chest XP at inspiration/expiration, and SNIF were measured every four months for two years.

Results: There were 16 patients (53.3%) in group A, 6 patients (20.0%) in group B, 7 patients (23.3%) in group C, and 1 patient (3.3%) in group D. In group A (upper limb onset), onset was on the right in 13 patients (81.3%), on the left in 3 patients (18.8%). In group B (lower limb onset), onset was on the right in 3 patients (50.0%), on the left in 2 patients (33.3%), and bilateral in 1 patient (16.7%). The mean (range) disease duration was 38.5 (16–101) months in group A, 44.7 (13–122) months in group B, 23.9 (10–48) months in group C, and the duration was 17 months in group D. When patients had common cold symptoms or intermittent subjective dyspnea, the abnormal values were detected in the following order: SNIF > DCMAP = %FVC > diaphragm movement on chest XP > blood gas analysis. There were some patients who did not perceive dyspnea even though diaphragm movement had disappeared. NIV was initiated during the study period in 9 patients in group A (4 patients for 24 h and 5 patients only at night time), 1 patient in group B (for 24 h), and 1 patient in group D (for 24 h).

Discussion and Conclusions: Previous reports have already shown that%FVC is inadequate as the criterion of initiating NIV. In the present study, abnormalities in SNIF were detected at the early stage of respiratory failure, indicating that they may serve as indicators of NIV initiation.

P175 TRANSCUTANEOUS PCO_2 MEASUREMENT IN ALS

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Keywords: transcutaneous PCO_2

Background: Respiratory insufficiency is critical for ALS patients, and spirography is usually used as the main method to investigate the respiratory function. It cannot, however, be applied so frequently, and sometimes fails to provide us with reliable data for accurate estimation of the breathing status in ALS patients with bulbar symptoms or elder patients.

Objective: An evaluation of the usefulness of transcutaneous PCO_2 ($tcPCO_2$) analysis for the assessment of respiratory function in ALS patients.

Methods: Nine patients with ALS were investigated. We measured $tcPCO_2$ and SaO_2 for 8 hours while the patients were asleep at night, and the examinations were repeated several times for each patient. Spirography and arterial blood gas analysis were also performed.

Results: Mean $tcPCO_2$ tended to increase with disease progression in 7 patients, and the fluctuation patterns of $tcPCO_2$ well corresponded to $PaCO_2$ on arterial blood gas analysis. In one patient on NIPPV, $tcPCO_2$ and SaO_2 improved after NIPPV was started. In the other patients, mean SaO_2 deteriorated, while mean $tcPCO_2$ improved.

Conclusions: With some dissociation between $tcPCO_2$ and SaO_2, $tcPCO_2$ seemed to generally reflect the respiratory functions in the ALS patients. As $tcPCO_2$ examination can be performed noninvasively for ALS patients in any condition, it seems to be a useful method to complement spirometric examination.

P176 EFFECTIVENESS OF MONITORING SNIFF NASAL INSPIRATORY PRESSURE (SNIP) AND TRANS CUTANEOUS PCO_2 TO FORESEE PROGNOSIS OF PATIENTS WITH ALS

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Keywords: SNIP, transcutaneous PCO_2 monitor, predict of prognosis of ALS

Background: Forced vital capacity (%FVC) is considered as the standard method to evaluate respiratory function of ALS patients. However, as the disease advances, technical difficulties increase to make it unreliable. We reported the effectiveness to monitor SNIP to foresee the ALS prognosis at this symposium last year. Meantime, trans cutaneous PCO_2 monitor has become available which is likely to serve the purpose.

Objectives: In this study we aim to 1) investigate effectiveness to monitor Trans cutaneous PCO_2 (TcPCO2) to foresee the prognosis, and 2)to compare SNIP and $TcPCO_2$ with the longitudinal observation.

Methods: 36 ALS patients were enrolled in the present study, whose SNIP was evaluated every $2\sim3$ months. Plug was inserted into nostril while contralateral nose was not occluded (opening nose method). In case of patients whose SNIP less than 30 cmH_2O, it was done while contralateral nose was occluded (occluding nose method). 6 healthy volunteers and 11 ALS patients were involved with $TcPCO_2$ monitor.

Results: During the study period, 11 patients had to be permanently ventilated (TPPV) or died. The logistic regression model shows that only average decrease of SNIP value (monthly decrease value /baseline SNIP) and baseline SNIP value contributed to poor prognosis (death or TPPV management) significantly whereas age, time since the onset and bulbar symptom did not. In event the baseline SNIP goes down less than 26.5 cmH_2O (81.8% for positive rate and 65.4% for negative rate) and/or the changes of SNIP value/month takes place to go down more than 7.05%,(72.7% for positive and 69.2% for negative) death or permanent ventilation results in most cases. (Kaplan-Meier survival curve and log rank test). $TcPCO_2$ suggests an increase of nocturnal pCO_2 in most cases who have low SNIP value. In a single case, SNIP is in normal range as well as daytime pCO_2 is normal but $TcPCO_2$ revealed CO_2 retentions in night time.

Discussion and Conclusion: Monitoring the baseline SNIP and changes of SNIP /month is the effective way to foresee prognosis. $TcPCO_2$ is possibly more accurate to foresee prognosis.

P177 APPLICATION OF POLYSOMNOGRAPHIC MONITORING IN PATIENTS WITH ALS

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Keywords: polysomnograms, oxygen desaturation, Sleep

Objectives: To evaluate the application of polysomnograms (PSG) in patients with ALS.

Methods: Twenty-one cases of ALS patients (9 females, 12 males), with age range of 24 to 78 years old (50.2 ± 10.8 years), were carried out PSG monitoring. The patients were divided into 1) bulbar group (9/21) and 2) limb group (12/21) according to the site of onset. The disease duration from the onset to the time of PSG ranged from 3 to 69 months (20.6 ± 17.9). PSG parameters included the maximum and minimum oxygen saturation in stages of NREM and REM. Sleep-related oxygen desaturation was determined by a dip in SpO_2 by more than 4% from the overnight baseline value. Polygraphic recording includes EEG (C3/A2, C4/A2), electrooculogram, chin and leg electromyogram. The respiration was monitored and measured by oronasal thermistors, uncalibrated inductive plethysmography indicating thoracic and abdominal efforts, finger pulse oximetry, and snoring sounds through a calibrated microphone.

Results: The results of arterial blood gases, pulmonary function tests, and sleep-related oxygen desaturation which was determined by a dip in SpO_2 by more than 4% from the overnight baseline value were classified and analyzed according to the site of onset of ALS. It showed that: 1) 3 patients had abnormal arterial blood gases in 21 patients (14.3%), the bulbar group had 1 patient (11.1%) and the limb group had 2 patients (16.7%). 2) 7 patients had their oxygen desaturation dropped more than 4% (33.3%) during the NREM sleep, among whom the bulbar group had 2 patients (22.2%) and

the limb group had 5 patients (41.4%). 3) 22 patients had their oxygen desaturation dropped more than 4% (52.4%) during the REM sleep, among whom the bulbar group had 4 patients (44.4%) and the limb group had 7 patients (58.3%).

Conclusions: Our preliminary results showed that PSG could detect the ventilation insufficiency happened in the absence of any symptoms of respiratory difficulty and abnormal arterial blood gases in ALS patients. Since it is suggested that abnormal response of motor neurons to hypoxia may participate in pathogenesis of ALS, initiation of respiratory care at an adequately early stage might improve the survival and the quality of daily life in ALS patients. Therefore, PSG should be included in a routine evaluation for ALS patients at an early stage to predict respiratory impairment.

P178 HELPING PATIENTS TO DECIDE ON THE BORDER OF LIFE: A VIDEO FILM ABOUT RESPIRATION, NONINVASIVE AND INVASIVE VENTILATION FOR ALS PATIENTS

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Keywords: ventilation, decision, video

Background: In ALS, respiratory deterioration can be treated by different methods: Non invasive ventilation (NIV), invasive ventilation via tracheotomy or palliative care with oxygen. Patient's autonomy should be supported by shared decision making. However, respiratory physiology and different types of ventilation support are difficult to explain to patients. We hypothesized that the additional use of a video clip may help to inform ALS patients better without relevant increase of anxiety.

Patients and Methods: We developed a 23 minute video clip explaining physiology and pathophysiology of respiration, non-invasive and invasive ventilation as well as palliative care in ALS. Film production was done by professionals. We tested the patients knowledge about ALS, respiratory failure and therapy a 25 item questionnaire which was answered before (Q1) and after (Q2) watching the movie. After the movie, we added questions about patients opinion about the video and about the probable influence of the video on the patient's decision.

Results: 55 ALS patients (mean age 56 (36–84) years) watched the video clip and completed Q1 and Q2. Knowledge about ALS and the management and effects of NIV and tracheotomy was improved in 49 patients. The remaining 6 patients were well informed before and completed Q1 without mistake. All patients judged the video clip to be informative or very informative. 8 of 55 patients felt anxious or fearful after seeing the video clip. Younger patients tended to experience the video more informative and less frightening. 30 of 55 patients thought the video to influence their further decision making. The majority of patients recommended to include the video clip into informed decision making about NIV, tracheotomy and palliative care in ALS.

Conclusion: Video supported information seems to be helpful for ALS patients to decide about the treatment of respiratory deterioration and terminal care. We propose to include such media into clinical practice in ALS.

P179 PERSPECTIVES OF PATIENTS WITH ALS ON THE IMPACT OF LUNG VOLUME RECRUITMENT THERAPY ON THEIR HEALTH AND QUALITY OF LIFE

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Keywords: rehabilitation, respiratory management, quality of life

Background: Lung volume recruitment (LVR) is a manual insufflation and cough augmentation technique used to help patients with ALS clear secretions. Although clinicians report positive results of LVR in terms of increasing lung volumes and improving cough effectiveness, limited disease-specific research data are available to support its use (1) and LVR has become a standard practice in several ALS clinics in Canada. In addition to the need for quantitative data on physiological outcomes, data on patient perspectives on the technique are necessary to inform clinical practice and quality of care.

Objectives: The purpose of this study was to examine patients' perspectives on the impact of LVR therapy on respiratory function and personal well-being utilizing a multi-item self-report questionnaire and a semi-structured interview.

Methods: Ten patients (8 with ALS and 2 with other motor neuron disorders) who had been doing LVR therapy for an average of seven months (SD = 5.3 months). The ALSFRS-R and the SWAL-QOL were used to characterize participants' functional status and quality of life related to swallowing and airway protection. Participants completed a 14-item, five-point Likert scale questionnaire related to airway protection and respiratory status. They also answered interview questions about their experiences with LVR. Questionnaire data were analyzed descriptive quantitative methods. Interviews were recorded, transcribed, and analysed using QSR Nvivo7 software, while following an interpretive description approach.

Results: Questionnaires: The majority of respondents (n = 10) agreed that LVR helped them to clear thick and thin secretions from their throat (77.8%). A large percentage of respondents (55.6%) felt that LVR helped them to keep their airway clear, to feel less anxious about their breathing, and to manage excess secretions. Five of the nine respondents indicated a positive change in cough strength after LVR, while 4 of the nine respondents indicated a positive change in voice quality after LVR. Interviews: Several themes emerged from the interview data and were consistent with questionnaire responses. Positive perceptions of LVR on physiological function were reported by n = 6, many of whom described their experiences in terms such as "good" or "excellent" and described improvements in secretion clearance and increased cough strength after LVR. Several participants (n = 4) reported feeling more in control of their respiratory health status and more capable of coping with episodes of respiratory infection. Positive perceptions related to personal well-being were also reported. Several participants (n = 5) also noted that LVR reduced their level of frustration related to their respiratory health status. The vast majority (n = 9) of interview participants agreed that LVR was worth their time and energy.

Discussion and Conclusions: Strong clinical and theoretical rationales exist for evaluating patients' perspectives to complement other outcome measures in the end-of life airway management of individuals with ALS. The findings of this study suggest positive effects of LVR on well-being and

respiratory function from the patient perspective. Further research should include analysis of patients' perspectives in relation to other outcomes of LVR and should include the perspectives of family members who are primary care providers.

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P180 THE EFFECTS OF MANUAL INSUFFLATION THERAPY ON THE SPEECH AND VOICE OF PATIENTS WITH ALS

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Keywords: Cough augmentation, speech, voice quality

Background: Over the past few years a simple and inexpensive treatment approach, lung volume recruitment (LVR), has been used to improve coughing and airway clearance of patients with ALS and other neuromuscular diseases. LVR is a manual breath stacking technique that involves the use of a resuscitator bag that has been retrofitted with a one-way-flow-valve and mouthpiece. Patients and/or their caregivers are taught to manually compress the resuscitator bag in a series of breath-stacking manoeuvres until the patient is maximally insufflated. Once patients reach maximum insufflation capacity, they are able to cough with sufficient force to clear their lungs and airways of mucus and other secretions. In ALS clinics where the LVR technique is frequently used, many patients have reported more normal voice quality and improved speech immediately after LVR treatment sessions.

Objectives: The purpose of this study was to assess the short-term effects of LVR on voice quality and to evaluate claims that LVR improves respiratory support for speech and "increases speaking volume" (1).

Methods: Eight patients with ALS previously trained in LVR were recruited to this pilot study. The research design was a one-group, pre-post test design (i.e., OXOO). Sustained phonation and connected speech samples were collected before and 30 minutes after a typical LVR treatment session. High-fidelity audio recordings were made with a MircoTrack 24/94 professional digital recorder (i.e., sampling rate of 44.1KHz with 16-bit resolution) and WAV files were analyzed using a KayPENTAX Sona-Speech II software package. A standard assessment of voice production (i.e., mean fundamental frequency, jitter, shimmer and mean decibel level) maximum sustained phonation and mean number of syllables per breath group was conducted. Changes in pre-post treatment were examined and comparisons to age-matched normative data were made. Inter-rater reliability judgments were performed on 30% of the overall sample.

Results: No changes were noted in overall pitch and loudness levels across the experimental conditions; however, a large effect was observed in post treatment jitter scores (i.e., a cycle-to-cycle measure of pitch variation that is considered the primary acoustical correlate of the perception of voice quality). Jitter scores improved from a mean baseline of 1.33% to 7.14% (as compared to norms of 21–27%). Maximum sustained phonation improved from a mean baseline of 13.03 seconds to 18.63 seconds (as compared to norms of 24.6 seconds). The average number of syllables per breath group produced while reading a standard passage improved from a mean of 11.73 syllables per breath group to 14.48 syllables per breath group (as compared to norms of 18.2 syllables). Overall inter-rater reliability was 88%.

Discussion and Conclusions: Preliminary findings of the effects of LVR on the acoustic and aerodynamic properties of speech and voice are positive. LVR may offer a short-term improvement in voice quality and respiratory support for speech. However, ffurther investigation of this therapeutic effect in a more controlled research experiment is necessary.

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P181 PROLONGED AND INTENSIVE MONITORING AFTER STARTING NON-INVASIVE VENTILATION IMPROVES TOLERANCE IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: amyotrophic lateral sclerosis, respiratory failure, tolerance to NIV

Background: The most frequent cause of death in patients with amyotrophic lateral sclerosis (ALS) is respiratory failure (RF) secondary to impairment of the respiratory musculature. In recent years, some studies have indicated that non invasive ventilation (NIV) improves survival and quality of life of ALS patients with RF. One restricting factor to the efficacy of NIV is bulbar involvement, but little is known about predictors of NIV adaptation and tolerance.

Objective: To evaluate the effect of prolonged and intensive monitoring in a multidisciplinary ALS clinic setting on tolerance to NIV in ALS patients with RF.

Patients and Methods: We prospectively monitored all consecutive ALS patients with RF who attended our ALS Center since June 2006. NIV was offered to 20 eligible patients (11 M and 9 F) according to current guidelines and it was initiated during a hospital stay in our ALS Center. Nine patients presented with severe ($n=5$) or mild-moderate ($n=4$) bulbar impairment. During the whole hospitalization period, all patients underwent a respiratory kinesitherapy program. Patients were assessed by: i) pulmonary function; ii) disability; iii) nutritional status; iv) quality of life. After discharge, all patients were regularly followed-up at three-months interval. According to accepted criteria, tolerance to NIV was defined as the ability to use the ventilator for more of 4 hours/day, for at least 60 consecutives days. NIV settings were adjusted as necessary during follow-up.

Results: The mean interval time for adaptation to NIV was 5 ± 2 days but the patients were allowed to prolong their hospital stay for an average extended period up to 3 weeks, during which a careful monitoring of NIV was performed. We observed that 19 of the 20 patients who initiated NIV, even those with severe bulbar impairment, remained tolerant at twelve months follow-up. One patient, with a moderate bulbar impairment, became non-tolerant to NIV three months after adaptation because of persistent airways mucus accumulation. Of the 19 tolerant patients, 11 (57.9%) had at least one change in NIV settings during the first year of follow-up.

Conclusions: Our study show that an intensive and prolonged monitoring in a hospital setting after NIV adaptation increases tolerance, even in patients with severe bulbar impairment. Moreover, a respiratory kinesitherapy during the NIV training and the NIV settings adjustments appear to further increase the tolerance.

P182 EVALUATION OF HOMECARE PROBLEMS IN HOME MECHANICAL VENTILATION USERS WITH ALS IN JAPAN

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Keywords: Home mechanical Ventilation, Homecare Problems, sufficient education

Background: The number of Home Mechanical Ventilation (HMV) users with ALS is increasing in Japan, but it is unknown about their problems with care at home.

Objectives: The purpose of this study was to evaluate the use of ventilators and accessory devices at home and identify problems in the management of care for HMV users with ALS.

Methods: Data were collected by requesting 684 organizations, including health centers all over Japan, to interview home-care "patients needing assistance for suctioning," and who consented to cooperate with the study. The interviews involved a patient profile, evaluation on the use of the respirator and accessory devices, and problems during the past year. Simple statistical processing of the data was performed, and the contents of the problems were analyzed.

Results: The survey covered 1331 consenting patients, including 749 home-cared ALS patients using a ventilator (56.2%). At the time of the survey, the mean period of ventilator use was 4.43 ± 3.91 (0–25) years, and the mean HMV period was 4.05 ± 3.69 (0–24) years, in the 749 patients. The method of ventilator use was TPPV in 687 (91.7%) and NPPV in 63 (8.4%), with 24-hour ventilation control in 672 (89.7%). Concerning accessory devices, 539 (72.0%) had an external battery, 733 (97.9%) had a suction machine, and 680 (90.7%) had a resuscitation bag. Problems occurred in 353 patients (47.1%), which were due to changes in the patient's condition in 201 (56.9%), and medical instrument malfunction in 144 (40.8%).

Discussion: The percentage of those undergoing NPPV was low in the 749 patients, probably because the patients were registered with public organizations, including health centers, which listed the patients in a serious state and the "need for suctioning." Duration of ventilator use was defined as the time after initiation of mechanical ventilation until HMV had been protracted. The finding that nearly half of the patients had experienced problems suggests the inadequacy of home care and the importance of sufficient education from the initiation of HMV and for as long as HMV is used.

Conclusion: This study revealed some aspects of HMV care in Japan. Since nearly half of the patients had experienced trouble during the past year, improvements in the home care environment for the prevention and early management of problems are considered to be vital.

P183 NON-INVASIVE VENTILATION AT THE END-OF-LIFE

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Keywords: non-invasive ventilation, end-of-life decisions, outcomes

Background: Non-invasive ventilation (NIV) has been accepted as the standard of care for patients with Amyotrophic Lateral Sclerosis (ALS) with respiratory insufficiency. While indications for initiation of NIV have been published, management of NIV and its withdrawal at end-of-life has not been evaluated.

Objectives: To determine whether NIV use persisted to time of death in patients followed at the ALS clinic at the University of Alberta who died in the period January 1 2004 to March 31 2008.

Methods: Deaths in this period were identified from the ALS clinic database.

Results: Of 133 deaths in this period, 54 (40.6%) died without attempting NIV, 9 (6.8%) were titrated, but did not continue, and 71 (54.1%) were titrated and used NIV. The NIV was not tolerated or abandoned by 5 (7%), withdrawn electively when death imminent in 6 (8.5%) and continued until death in 60 (84.5%)

Conclusions: We conclude that that the majority of those who initiate NIV continue its use until death, and do not withdraw before death. Optimal management of patients using NIV at end of life is not yet reported.

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THEME 10 MULTIDISCIPLINARY CARE AND QUALITY OF LIFE

P184 ALS OUTCOME MEASURES: INSENSITIVE TO EARLY CHANGE?

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Keywords: FVC, MMT, ALSFRS-R

Background: Different quantitative measures have been used in clinical trials, but there is no consensus as to which tests most sensitively and reliably detect a modest drug effect in ALS. Most trials have included a pulmonary measure, and many have included the ALSFRS-R and a strength assessment. A better understanding of the strengths and limitations of outcome measures is important.

Objectives: To assess the relative sensitivity of FVC, MMT, and ALSFRS-R to early decline in ALS. To determine whether selecting patients who show early decline can increase study power.

Methods: We used data from 206 placebo patients enrolled in a clinical trial of minocycline. All had monthly measurements of FVC, MMT, and ALSFRS-R. We calculated the percentage of patients who had values that did not change from the baseline visit to months 1, 3, 6 and 12. We also investigated whether criteria, such as a maximum MMT score, could be used to define a subset of patients showing definite progression during the first 4 months following enrolment, and the effect of selecting only these patients on sample size estimates.

Results: 1) The ALSFRS-R had the highest percentage of patients with non-declining scores. At one month, 48**%** of patients had the same score or higher than at enrolment; the values were 33**%** at 3 months and 17**%** at 6 months.

2) For % FVC, 42% patients had $<1\%$ change at one month, 25% at 3 months, and 17% 6 months.

3) MMT showed the smallest percentage of non-declining patients. After one month, 41**%** failed to decline in strength, 27% at two months and 14% at 6 months had not declined.

4) Change in FVC correlated with the ALSFRS-R total score, but not with the respiratory subscale.

5) At 12 months the non-decliners (among those still being followed) were ALSFRS-R 4% (6/164), FVC 10% (12/118), MMT 12% (14/119)

Discussion and Conclusions: These data show the early insensitivity of 3 commonly used outcome measures. Although the 12 month sensitivity to change is higher than months 1 to 6 in all three measures, the lack of early change negatively impacts the best fit slope used to determine drug effect in clinical trials. Restricting trial inclusion to patient subsets showing early decline in the quantitative measures did not improve study power in our analysis. The need for additional strength and function assessments that are more sensitive to early change is apparent. Further study is needed to define how much insensitivity is due to the measurement tools, and how much is due to a subset of ALS patients who have only modest decline early in the disease.

P185 FEASIBILITY OF INTENSIVE CONTROLLED EXERCISE IN THE EARLY STAGES OF AMYOTROPHIC LATERAL SCLEROSIS (ALS-ICE), A PILOT STUDY

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Keywords: Exercise, Intensive, Aerobic

Background: There is ongoing debate whether exercise will improve the independence of patients with ALS or whether exercise speeds up the deterioration. In addition, there is insufficient evidence to suggest which type and intensity of exercise, if any, would be best for patients with ALS.

Objective: To conduct a pilot study which assesses the feasibility of an intensity controlled exercise programme for patients with ALS.

Methods: Five patients conducted a moderate-intensive aerobic exercise programme of one hour of exercises, twice a week, during a period of twelve weeks. Outcome measures included aerobic capacity (VO_2max with Astrand-Rhyming test), functional health status (ALSFRS-R) and strength, Maximal Voluntary Isometric Contraction (MVIC). The MVIC was based on 7 bilateral muscles (4 arm and 3 leg). For each patient a mean z-score was calculated. Side-effects were measured by registering injury reports of the patients and by measuring fatigue on the Checklist Individual Strength, subscale fatigue severity (CIS-fatigue). Intensity of the exercise program was registered by monitoring heart rate and Rating of Perceived Exertion (RPE 0-11) in training-diaries. Measurements were planned before and after the 12 week training period.

Results: Four males and one female with ALS participated in the pilot study. Mean age was 47.6 years (range 32–56, SD 9.3). Mean ALSFRS at study onset was 50.6 (SD 2.2) All five patients had been diagnosed with probable or definite ALS according to the El Escorial criteria.

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Intensity controlled exercise: Patients performed aerobic exercises between 55–70% of HRmax and RPE 3-5. Strength endurance exercises were set at 3 series of 12–15 repetitions (RPE 3-5).

Outcomes: All patients demonstrated a decrease in VO_2max (ranging from −8 to −67%). Functional Health Status did not change during the training period (change in ALSFRS-R ranged from +1 to −3). Muscle strength deteriorated in all patients (range −0.05 to −0.88)

Side effects: Only one patient experienced more fatigue on the CIS scale after the period of the training. No other side effects were reported.

Patients' experiences: All patients completed the training and tolerated the programme very well. They judged intensity as 'intensive' but not 'too intensive'. All patients would recommended the training to other patients with ALS and all of them continued the training on completion of the study.

Conclusions: Intensity controlled exercise is feasible and appears to be safe in patients with ALS. A large multicenter trial will start this fall.

P186 INTERNATIONAL CLASSIFICATION OF FUNCTIONING, DISABILITY AND HEALTH (ICF) CORE SETS FOR AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: ICF, functioning, disability

Background: Functioning is increasingly being taken into account for evaluating disease impact on the person living with amyotrophic lateral sclerosis (ALS) and drug treatment/rehabilitation effectiveness. With the International Classification of Functioning, Disability and Health (ICF), we can now rely on a globally-agreed-upon framework and system for classifying the typical spectrum of functioning problems of people with ALS, given the environmental context in which they live. ALS ICF core sets are lists of ICF categories selected to identify those aspects of functioning that are most likely to be affected by ALS.

Objective: To identify the preliminary comprehensive and brief core sets for ALS using the ICF approach.

Methods: Focus groups and a consensus process were used to identify ICF core sets for ALS, including: preliminary ICF studies; empirical patient data collection for 53 ALS participants and review of the evidence in ALS literature.

Results: One hundred and fifty-one second level ICF categories were selected. The comprehensive ALS ICF core set includes 38 categories from the component 'body function', 5 categories from body structures', 64 from 'activities and participation' and 44 from the component 'environmental' factors. Eleven categories in 'personal factors' in ALS were also suggested by the participants after intensive discussions. The brief set comprises 23 categories, 15% of categories in the comprehensive core set.

Discussion and Conclusions: Consensus expert opinion can use ICF categories to identify the core set for ALS which reflects disease complexity and care burden for people living with ALS. Further research is needed to identify ICF categories of relevant personal factors to improve our understanding of the large social and cultural variance associated with them.

P187 PROBLEM-FOCUSED CHECKLIST FOR PATIENTS AT AN ALS MULTIDISCIPLINARY CLINIC

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Keywords: Patient care checklist

Background: Multidisciplinary clinics are the standard for the care of people with ALS. When patients come to an ALS multidisciplinary clinic for routine quarterly visits, they are seen by multiple clinicians. Patients usually present with different problems they want to discuss and there is often a need for some services over others. Too often, these are elicited late in the visit and the particular experts needed for these problems do not get enough time with the patient.

Objective: The aim was to develop a brief checklist to provide to patients in advance of their scheduled visit, asking them to check the problems and issues they wish to discuss. The goal was to provide more focused consultations in order to address each patient's concerns, and to improve the flow of the multidisciplinary team.

Method: The checklist has 2 parts. Part 1 lists 16 problems, both medical and psychological. Items include difficulty breathing, drooling, weight loss, fatigue, depression, and memory problems. Part 2 enumerates 9 services that the patient may seek, such as advice about advance directives, how to arrange for a home health aide, questions about equipment, PEG, and hospice.

Results: To date, 80 patients seen consecutively have completed the checklists. 57% were male, and mean time since diagnosis was 17 months. Mean ALSFRS score was 33 (7–46) and mean FVC was 67 (13–119). Median number of problems reported was 3 or 4. In Part 1, patients most often wanted help with limb weakness (71%), fatigue (50%), and difficulty speaking (43%). Depression, anxiety and memory problems were endorsed by only 18%, 14%, and 13%, respectively. In Part 2, the most commonly reported issues concerned assistive devices (34%), exercise (28%) arranging for a home health aide (23%) and advance directives (15%). Least endorsed were questions about relationship/sexuality issues (3%) and hospice (5%). Patients with more advanced disease (lower ALSFRS and FVC) checked more problems and issues, and the numbers of problems and issues endorsed were themselves correlated.

Conclusions: The checklist strategy was well received by patients, who appreciated being treated as partners in their care. The range of problems and issues identified indicates the need for the expertise of a broad range of specialists. In a multidisciplinary clinic where multiple specialists are available to see several patients on the same day, checklists appear

helpful in allocating staff time to address specific needs, and high-need patients can be identified in advance. Data collection is ongoing, and if these patterns persist, clinic procedures can be revised accordingly.

Acknowledgement: Eleanor & Lou Gehrig MDA/ALS Research Center team.

P188 DESCRIPTIVE AUDIT OF THE KEY CHARACTERISTICS OF THE CURRENT MND POPULATION IN A LARGE UK SPECIALIST MND CLINIC

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Keywords: audit, patient characteristics, specialist clinic

Background: A specialist service has been established at this centre for many years, providing on-going follow up for people with MND. As part of a larger study exploring the personal experience of living with MND, an audit of the patient population was undertaken to identify prevailing characteristics of patients currently followed up by the service.

Objectives: The aims of this study were to determine the personal demographic characteristics of the patient population; identify site/type of disease onset and current severity (using the ALS Health Severity Scale (ALSHSS) (1) and ALSFRS-R, (2); assess level of uptake of PEG, NIV and aids/equipment; assess level and type of care required; determine the time from disease onset to diagnosis and identify the time from diagnosis to nurse specialist follow-up.

Methods: A comprehensive case note review was undertaken of patients (n = 98) currently receiving follow up at this centre. Statistical analysis was aided by SPSS version 14.0 to produce descriptive statistics indicating frequency of occurrence of key characteristics.

Results: The mean (standard deviation, SD) age at onset was 59.7 (12.6) years. 54.1% of the patients were male. Most patients (38.8%) had ALS with limb onset, with 29.6% having ALS with bulbar onset. The majority of patients were classed in the level 2 category of the ALSHSS, (58.2%) compared to 17.3% in level 1, 18.4% in level 3 and 6.1% in level 4. Four patients (4.1%) were on NIV and 16(16.3%) had a PEG inserted. The majority of patients relied on 1–3 items of equipment (46.9%) compared to 33% who used more than 3 items. 16.3% of patients did not currently use any aids or equipment. 28.6% of the patients lived independently with no uptake of care. 23.5% lived independently but with family assistance and 19.4% relied on family carers full time. 18.4% relied mainly on family but also accessed social services care. 5.1% of patients lived in residential accommodation. The mean time from symptom onset to diagnosis was 18.7 months (SD = 15.2) with 41.7% of patients receiving their diagnosis within 12 months of symptom onset. The majority of patients (78.5%) were commenced on nurse specialist follow up within 12 weeks of diagnosis with the mean length of time from diagnosis to commencement being 6.3 weeks (SD = 11.2).

Conclusions: This audit demonstrates the characteristic features of the current MND population followed up by this centre and for the first time provides detailed information regarding the level of disability and uptake of care services and equipment amongst this population. The length of time from symptom onset to diagnosis for most of the patients at this centre corresponds with evidence of a UK average of 11–22 month delay (3).

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P189 CHARACTERIZATION OF PATIENTS SUFFERING FROM MOTOR NEURON DISEASE IN DENMARK

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Keywords: Clinical profile, diagnosis

Background: Motor neuron disease (MND) consists of a group of neurodegenerative disorders including progressive muscle atrophy, amyotrophic lateral sclerosis (ALS) and primary lateral sclerosis. In Denmark these disorders are mainly diagnosed by neurologists in the ALS-teams located at 14 different hospitals. The Danish ALS-register was established in 2003 and until now 8 ALS-teams have included all their MND-patients. The register consists of two parts 1) the diagnostic process and 2) essential data in the course of the disease such as FVC, BMI, ALS-FRS as well as data about aids, nurturance needs and the patients preference for PEG-tube and NIV/IV. All data were collected in the out-patient clinics and from home visits performed by the Rehabilitation Centre for Neuromuscular Diseases.

Objectives: To characterize the clinical profile of the ALS-patients in Denmark and to find out how the diagnosis was reached and which investigations were performed.

Methods: This prospective study included 252 patients with MND diagnosed according to the El Escorial revised criteria from 2003 until 2008. The data collection included information on age, sex, site of first MND symptoms, time from first symptom to diagnosis, the diagnostic process and the certainty of the diagnosis.

Results: 252 patients with sufficient information were analyzed. Of them 146 were men (57.9%) with a mean age at first symptom 59.2 ± 12 and 106 were female (42.1%) with a mean age 61.2 ± 11.7. 19 cases (7.5%) had a positive family history of MND and in one of these cases a mutation in the SOD1 gene was found. The frequency for the site of first MND symptom were lower limbs (36.9%), bulbar region (35.7%), upper limbs (34.1%), respiratory symptoms (1.9%) and cognitive problems (1.6%). At the time of diagnosis the frequency of MND symptom were upper limbs (77.7%), lower limbs (72.2%), bulbar region (65.9%), respiratory symptoms (34.9%) and cognitive problems (6.0%). The diagnostic foundation at inclusion were history, clinical signs, EMG (90.1%), MRI of cerebrum and medulla (48.0%), lumbar puncture (39.3%) and measurement of cerebral blood flow (2.0%). The certainty of the diagnose according to the revised El Escorial criteria were definite ALS 41.7%, probable ALS 18.7%, probable ALS laboratory supported 5.6%, possible ALS 6.0%, suspected ALS 17.9% (LMN signs only 15.1% and UMN signs only 2.8%) and unknown 10.3%. The mean delay between onset of symptoms and diagnosis for the total study group was 16.4 ± 13.2 months

A previous, unpublished, study with ALS patients from Copenhagen has shown that more than half of the patients chose PEG-tube when swallowing problems occurred, but only a few patients in this area wished for or chose NIV or respirators. From January 1998 until January 2008 only 8 out of more than 135 ALS patients in the central area of Copenhagen chose NIV and another two ALS patients were ventilated with a respirator.

P190 NON NEUROLOGICAL MULTIDISCIPLINARY CARE IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS IN SPAIN

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Keywords: Multidisciplinary care, Non neurological care, Spain

Background: The health care of ALS patients depends on adequate knowledge of the disease and the resources of the health care system. Identification of care deficits, caused by failures of the system or the professionals, is the first step needed to resolve them.

Objectives: To determine the health care status of Spanish patients with ALS, specifically their non neurological multidisciplinary care in order to identify needs and to propose improvements.

Methods: We created a 156 item questionnaire that included demography, clinical status and administered health care. Of those, 73 questions related to non neurological multidisciplinary care. The questionnaire was hand delivered or mailed to up to 400 patients with ALS from the Spanish ALS Foundation nationwide. Response to date is 260. A univariant analysis with SPSS 10.0 for Windows was performed.

Results: The patients median profile is male/female (ratio 1:1); 54 years old (range 29–79); married (81%); spouse as primary caregiver (70.8%); and from Madrid (41.5%). Specific care habits and deficits were identified. Among those, 47.6% of patients did not receive any form of physiotherapy; and in the rest of patients the physiotherapy was limited and insufficient. Between diagnosis and prescription of physiotherapy there was a median delay of 9.7 months; the mean duration of therapy was 1.8 months. 25.6% of patients received it at private centers and 28.2% at their homes, all of them at their own cost. Specific technical aids needed and not provided were identified. Patients financed 73.1% of simple technical aids and 100% of advanced aids. 73.5% of patients with dysarthria did not receive speech therapy. Of those receiving it, 36.4% had it at a private center and 36.2% at their own home, all of them at their own cost. 72.4% of patients with dysphagia did not receive nutritional information or care. 56.4% of patients with respiratory insufficiency do not have any ventilatory assistance. Specific technical aids needed and not provided were identified. 75.0% of patients with emotional distress did not receive psychotherapy. 60.2% were taking psychotropic drugs. 43.2% of patients did not receive the home assistance needed. Detailed analysis in relation to clinical status will be presented.

Conclusions: There are very significant deficits in physicotherapy, technical aids, speech therapy, nutritional care, ventilatory assistance, psychotherapy, and home care. The solution of these deficits requires the creation and formation of true multidisciplinary and coordinated care teams in each large hospital. Public aid to finance technical aids for mobility and communication is required.

P191 THE REVISED ALS FUNCTIONAL RATING SCALE: VALIDATION OF AN ADAPTED VERSION TO THE SPANISH ALS POPULATION

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Keywords: ALSFRSr, Validation, Spain

Background: The revised ALS Functional Rating Scale (ALSFRSr) is a widely validated instrument to measure disease progression in ALS. The ALSFRSr contains 12 items grouped into four domains (gross and fine tasks, bulbar and respiratory functions) which grade disabilities in activities of daily life (ADL). It has been used as a primary variable in several recent clinical trials. Clear understanding of each item scoring is necessary to avoid technical errors. Cultural differences make it necessary to validate any translation of the original English version into another language.

Objectives: To validate an adapted Spanish version of the ALSFRSr.

Methods: The ALSFRSr was translated into Spanish. Two raters carried out the evaluation of 60 patients with probable or definite diagnosis of ALS according to El Escorial Criteria. The items of the Spanish version of the ALSFRSr were correlated with the functional variables of the ALSAQ-40 quality of life questionnaire (physical mobility, ADL and independence, eating and drinking, and communication) and the respiratory variables of the SRI (Severe Respiratory Insufficiency) questionnaire (respiratory symptoms, physical function, and sleep). Internal consistency was examined by the Cronbach statistical alpha. Test-retest correlation analysis was made using the Spearman's rho. A factor analysis, with extraction of main components with Varimax rotation and Kaiser normalization, for comparison with the original scale was performed. Correlation between the ALSFRSr dimensions and the corresponding ones of the ALSAQ-40, and between the ALSFRSr respiratory dimension and the SRI questionnaire was obtained for validation.

Results: Cronbach's alpha values were between 0.75 and 0.95 for all dimensions to prove internal consistency. Spearman's rho values were between 0.80 and 0.95 for the test-retest analysis. The factor analysis showed a contribution of nearly 55% of the principal component; the load of each item to its correspondent factor was between 0.50–0.90 except for the "salivation" item. The structure of the scale was found, through the Varimax rotation, to be divided into 3 factors: neuromuscular, respiratory and bulbar functions. The construct validity revealed strong correlations ($r_s > 0.60$) with the ALSAQ-40 between Gross Motor task and Mobility, between Gross and Fine Motor tasks and ADL, between Bulbar Function and Communication and between Bulbar and Respiratory and Eating and Drinking. Similar index correlation was obtained between the ALSFRSr respiratory dimension and its corresponding ones of the SRI.

Conclusions: According to these results, the Spanish version of the ALSFRSr is a highly reliable and valid instrument for the functional assessment of Spanish patients with ALS.

P192 INTERDISCIPLINARY CARE IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Keywords: multidisciplinary care, nutrition, dysphagia

Background: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease, causing progressive weakness and loss of the muscular strength of superior and inferior members subsequently compromising, the respiratory system. Dysphagia, weight loss, dyspnea and depression, besides limitations of the activities of daily living (ADL), are difficulties presented with the progression of the disease. The integration of the informations is the benefit of the interdisciplinary care, providing better quality of life to patients and caregivers.

Objective: To study the effectiveness of the interdisciplinary care in patients with MND/ALS.

Method: This prospective non-randomized study was carried between June 2007 and February 2008 in 33 patients with ALS, who were regularly receiving interdisciplinary care and were following the therapeutic protocols. Specialties included were neurology, respiratory physiotherapy, speech and language therapey and nutrition. The period of evaluation was quarterly and patients were assessed for medical treatment, nutritional status, dysphagia, forced vital capacity (FVC) and use of BiPAP. For statistical analysis test of Wilcoxon was used.

Results: The average age was 55,0 years, ranging from 33 and 82 years; 20 (60.6%) was male and 13 (39.4%) female; 84,8% were using Riluzole. Tamoxifen was associated in 36.7% and 39.4% were taking E and C vitamins. Complex B was used in 6.0%. In spite of the nutritional orientations, 12.0% (4) presented with malnutrition. Twelve (36.3%) had PEG and 21 (63.3%) had oral feeding. The majority (54.4%) did not change their food consistency, 42.4% were ingesting a creamy diet and 6.0% were receiving nutrition exclusively by PEG. During the study, only 21.1% of the patients presented exchange of food consistency, while 33.3% experienced increasing dysphagia; 33.0% did not use BiPAP despite necessity (FVC reduction nocturnal and respiratory compromise and/or during the day). The values of FVC varied between 17,0% and 84,0%. Despite necessity, respiratory physiotherapy is not even assessed in the same proportion of those adhering to the medicine and to the changes in the diet. We didn't observe differences in the nutritional status, feeding route, dysphagia intensity, use of BiPAP and measure of FVC. We obtained statistically significant difference ($p = 0,11$) only for food consistency.

Conclusion: The interdisciplinary care brings adherence to the treatment of the MND/ALS. However, the application of the given orientations depends of the availability of the patient and caregivers.

P193 FIRST IMPRESSIONS COUNT-SATISFACTION AT THE TIME OF DIAGNOSIS PREDICTS PERCEIVED LEVEL OF SUPPORT RECEIVED AT PRESENT IN MND PATIENTS

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Keywords: Diagnosis, satisfaction, survey

Background: The Motor Neurone Disease (MND) service is based in the Walton Centre for Neurology and Neurosurgery in Liverpool. The service covers the North West of England, Wales and the Isle of Man with a combined population of 3 million people.

Objectives: The aims of the present study are twofold 1) to evaluate which elements of diagnosis are most strongly predictive of a patient's level of support with the service provided at diagnosis, and 2) to measure whether the patients' support level at diagnosis is predictive of their satisfaction with their present support.

Methods: The MND database at the Walton Centre was used to identify potential participants. Patients too ill to participate, who had opted not to be contacted by the service for research or had a known history of dementia were excluded from this postal questionnaire survey. 142 out of 170 patients on the database were contacted. Perceived support at the time of diagnosis was measured as excellent, good, satisfactory or poor. Access to information and health professionals at the time of diagnosis, and satisfaction with services at the time of participation in the survey were used as predictors of perceived support.

Results: The response rate was 54 out of 142 (38%).

With regard to patients' view on the service at the time of diagnosis, 13% rated it as poor, 20% as satisfactory and 66% as good or excellent. Elements of diagnosis that were assessed included meeting an MND specialist nurse around the time of diagnosis and being given information on the MND Association.

Two elements of diagnosis were predictive of a patient's level of satisfaction with the service. Patients informed about the MND Association rated the support received at diagnosis as excellent or good 78% of the time, versus 11% of those who were not informed. Patients meeting the MND specialist nurse rated the support received at diagnosis as excellent or good 89% of the time; in contrast only 26% of those who did not meet the nurse rated support as good or excellent.

A short scale measuring current level of support was constructed with a maximum score of 9 (absolute satisfaction) and a minimum of -9. Patients who rated their support at diagnosis as 'excellent' were the most satisfied and those who rated their level of support as 'poor' the least.

Discussion: Explaining about the MND Association leads to greater levels of perceived support at diagnosis. The importance of early contact with an MND specialist nurse is also highlighted. We suggest that people who feel supported and engaged with current services report better disease-specific input at diagnosis.

P194 IDENTIFICATION OF COPING STRATEGIES USED BY CARERS OF FAMILY MEMBERS WITH A DIAGNOSIS OF MOTOR NEURONE DISEASE – A QUALITATIVE STUDY

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Keywords: Motor Neurone Disease, Carers, Coping strategies

Background: Caring for a person living with a progressive illness, such as Motor Neurone Disease is acknowledged as both challenging and demanding (1). The demand of caring for a person living with Motor Neurone Disease can become a source of stress for informal carers.

Despite literature identifying coping strategies used by carers of individuals with conditions such as stroke or cancer, it was difficult to identify coping strategies used when caring for a family member with Motor Neurone Disease. Therefore the Author felt that this study, as part of an MSc in Rehabilitation, would provide fruitful data by exploration of the thoughts and experiences of these individuals.

Objectives: The key objective of the study was to identify coping strategies used when caring for a family member with Motor Neurone Disease.

Methods: Ethical approval was obtained. A qualitative method was chosen for the study. Purposeful and convenience sampling was used. Six participants agreed to participate in the study, (three female and three male). Semi-structured, face to face interviews were carried out using an interview guide. Data analysis was carried out by using a thematic content analysis method (2).

Results: The following themes were identified:

- Category one – Support for carers.
- Category two – Emotional Coping Strategies
- Category three – Protected Time for Carers
- Category four – Carer's Role

Discussion and Conclusions: The findings were comparable to that of existing evidence, which used different patient groups within the study population such as individuals effected by cancer. The semi-structured interviews within this study allowed the generation of data providing an insight into the carer's personal experiences.

Implications for Future Practice: The following actions could be taken i) Development of information leaflet highlighting coping strategies. ii) Gauge how useful carers would find a MND specific carer support group, with view to setting such groups locally

A number of areas for future research were identified: i) Longitudinal study identifying coping strategies used from diagnosis. ii) Comparison study of support services involved in the management of MND in Lincolnshire and Cambridgeshire and iii) Compare the coping strategies used by family carers of individuals with a diagnosis of MS and MND

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P195 DIFFERENCES IN BURDEN AMONG CAREGIVERS OF PEOPLE LIVING WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: CBI, quality of life, support

Background: Caregivers of patients with Amyotrophic Lateral Sclerosis (ALS) have to cope with several practical difficulties together with the suffering of people who usually are very close and intimate to them. Therefore, we need to assess the amount of psychological distress caregivers commonly have to face, taking into account how such a distress can be devastating for their health and quality of life. Moreover, in terms of intervention, working for caregivers' well-being can exert a positive effect on patients' quality of life too.

Objective: We aimed to evaluate caregivers' burden, in relation to variables such as sex differences among caregivers and ALS patients' physical impairment due to disease progression.

Methods: We used the Caregiver Burden Inventory (CBI), a 24-item questionnaire based on 5 subscales measuring different aspects of caregivers' burden. We administered CBI to 31 caregivers of ALS patients and we divided the sample in three age groups (Age 1: 26–45; Age 2: 46–59; Age 3: 60–70) and in three classes identified by the patients' ALS Functional Rating Scale scores (Class 1: 36–48; Class 2: 25–35, Class 3: 0–24). We correlated different CBI dimensions with caregivers' age, sex and disease severity.

Results: Female caregivers scored significantly higher in the subscales related to time for assistance, physical burden and sensation of not having the same opportunities of people of the same generation. As far as the "age" variable is concerned, we only found a significant difference in physical burden, that-as one could expect-was higher in the eldest caregiver (Age 3 Group), in comparison to that of the youngest (Age 1 Group). Another finding concerns the significantly higher time caregivers of the most physically impaired patients have to spend giving assistance: statistically significant differences on this CBI dimension were observed between Class 3 and Class 1 and also between Class 3 and Class 2.

Discussion and Conclusions: Our data can foster further investigation on caregivers' burden. We found some interesting differences among caregivers which could be of great relevance in setting of suitable person-centred psychological intervention: caregivers should be regarded as people who need support and at the same time, subjects playing a key role in patients' support.

P196 RELIGIOSITY DOES NOT INFLUENCE QUALITY OF LIFE AND DEPRESSION IN ALS CAREGIVERS

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Keywords: Caregivers, religiosity, depression

Background: It has been demonstrated that religiosity has a profound effect on ALS patients outcome (1) and quality of

life (QoL) (2). However, there are no studies on the influence of religiosity on caregivers of ALS patients.

Aim: To assess the effects and correlates of religiosity on ALS caregivers.

Methods: A total of 75 consecutive informal caregivers of ALS patients were interviewed. Religiosity was evaluated using the Idler Index of Religiosity (IIR), a four-item measure consisting of two items assessing public religiosity and two items assessing private religiousness. Total scores range from 4 (least religious) to 17 (most religious). QoL was evaluated with McGill Quality of Life (MQOL) questionnaire, and Satisfaction with Life Questionnaire (SWLQ). Depression was evaluated with Zung Depression Scale (ZDS) and anxiety with the Spielberger's State and Trait Anxiety Inventory (STAI). Correlations between religiosity and the other variables was performed with Pearson's correlation; multivariable analysis was performed with linear regression.

Results: The 75 caregivers (26 men and 49 women) had a mean age of 55.8 years (SD 12.0). They included 47 spouses, 16 children, 5 parents, 3 siblings, 2 nephews, 2 brother/sister-in-law. Caregivers' mean IIR score was 10.7 (SD 3.0) (private 5.9 (SD 2.1), public 4.8 (1.3)). Their mean ZDS score was 39.3 (SD 8.1); 6 (8%) caregivers had a score above the depression cut-off:. Caregivers' mean MQoL score was 7.4 (SD 1.3), their mean STAIs score was 48.1 (SD 11.7); state anxiety level was low in 19 (25.7%) caregivers, medium in 44 (59.4%) and high in 12 (16.2%). Caregivers' mean SWLS score was 25.0 (SD 6.9). In multivariable analysis caregivers' MQoL was related to SWLS score ($p = 0.001$) and ZDS score ($p = 0.02$) but not to total IID, private IID or public IID. Private IIR was related to caregivers' age ($p = 0.006$) and education ($p = 0.047$), while public IIR was related only to caregivers' education ($p = 0.021$).

Conclusions: Differently from patients, religiosity had no relevant influence on caregivers' QoL, which was conversely related to Satisfaction with Life and depression. The reasons for these discrepancies should be investigated further.

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P197 HOW AWARE ARE CAREGIVERS OF THE CONSEQUENCES AND MANAGEMENT OF BULBAR INVOLVEMENT IN ALS?

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Keywords: caregivers, bulbar involvement, speech therapy

Background: The important role of **ca**regivers in ALS is increasingly recognized. The bulbar involvement needs the speech therapist to work on several aspects of this deficit: dysphonia, dysarthria, dysphagia, tongue and cheeks paresis and emotional lability. His role is not restricted to the patient, as informations must be given to the caregiver. These caregivers have to be informed of several aspects concerning dysphagia or dysphonia and their consequences. They also must learn how to behave in case or emergency.

Objectives: We tried to determine how much and how correctly the caregivers in bulbar ALS cases are informed.

Material and Methods: During a 30 minute interviews, caregivers were asked to answer a questionnaire about the current speech therapy at home, dysphagia, food intake, communication and also about the information they had received simce the beginning of ALS. Each interview took place in our center during the quarterly multidisciplinary consultation.

Results: There were 45 caregivers, 69% were spouses of the patients and mean age was 59.2yrs. 91% of the corresponding patients had begun speech therapy and for 98% of them more than once a week. For 57% speech therapy was done at home. For only 45% of the patients, the caregiver was present during the speech therapy session.

Dysphagia: 40% of the caregivers did not know the appropriate posture for swallowing, only 29% of them would be able to perform a Heimlich Maneuver and 36% of them had never heard of it. *Food intake:* Only 44% of the caregivers were aware of the usefulness of mixed food, 14% were informed about water jelly while 76% recognized that the patient had feeding troubles. *Communication:* 37% had frequent difficulties in understanding the patient's talk and 18% never understood. Almost 16% of the caregivers never used written communication. *Information:* 69% of the caregivers considered their own information about communication tools as insufficient. 64% felt the same about gastrostomy. For 60% of the caregivers, better information and better knowledge would help them to be more efficient in providing patient care.

Discussion: This work underlines huge gaps in the knowledge of caregivers about ALS and the specific deficits of the patient they care for. For bulbar involvement, the speech therapist may help improve such knowledge. However, it seems important to draw the attention of the caregiver on the specific deficits induced by ALS during specific sessions at the expert center. This work may help physicians and expert teams pay particular attention to those points and to create training and information sessions devoted to caregivers as early as possible in the disease course.

P198 THE LONG TERM EFFECTS OF SALIVARY GLAND RADIATION THERAPY ON SWALLOWING AND MEALTIME RELATED QUALITY OF LIFE: A CASE STUDY

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Keywords: Sialorrhea, swallowing, quality of life

Sialorrhea is a common bulbar symptom in patients with ALS that results in significant functional, social and psychological burden on patients, families, and caregivers. Neuromuscular induced weakness and wasting of the lips, tongue, pharynx and larynx results in open mouth postures, poor oral containment of thin salivary secretions and ineffective swallowing. Most research on sialorrhea has focused on the anterior spillage of saliva out of the mouth (drooling); limited data exist on the impact of posterior spillage of saliva into pharynx, which is particularly problematic in bulbar cases of ALS. As secretions spill into the pharynx, they place constant demands on airway protective mechanisms. Frequent coughing, gagging, and discomfort at mealtimes can have a profound impact on quality of life. If patients are unresponsive to pharmacological interventions or these are contraindicated, other approaches such as salivary gland radiation therapy (XRT) may be used. Positive results have been reported in the few treatment outcome studies that exist on XRT however most studies to date have focused on quantifying saliva production following treatment (e.g., drooling collection units, tissue counts) and limited data are available on patient

perspectives on the impact of XRT on swallowing function and mealtime related quality of life.

Objectives: The purpose of this case study was to describe the long-term effects of salivary gland radiation therapy on swallowing and mealtime related quality of life for one patient with ALS.

Methods: A 29 year- old male with a bulbar onset of ALS received an increasing dosage of amitryptiline over 12 months and presented with moderate oropharyngeal dysphagia and severe sialorrhea including skin irritations around nose and upper face, clothing changes 2–3 times daily, with excessive salivary issues noted at mealtimes and while sleeping. He received two courses of radiation therapy over 5 weeks (i.e., five bilateral parotid gland treatments and five submandibular treatments). The Salivation Scale; Degree of Drooling Scale, The Saliva Control Assessment, the Post-Saliva-Surgery Assessment Form (1) the ALS-FRS and the SWAL-QUAL swallowing related quality of life instrument (2) were administered prior to XRT and then one month and thirteen months after treatment.

Results: Prior to XRT he stated that the effect of sialorrhea on his quality of life was severe and after XRT all saliva management issues were eliminated and the patient had a sufficient quantity of saliva to maintain more normal swallowing. He also reported improved psychosocial function at mealtimes. After XRT, he reported a decreased in overt symptoms of swallowing difficulty (episodes of coughing, choking, gagging, etc.). Additionally, his perceived level of psychological distress at mealtimes improved by 70%. His perception of these functions remained at this elevated level a year later across multiple measures.

Discussion and Conclusions: Saliva management, swallowing and airway protection are integrally related functions that affect quality of life.Results from this case study will form the basis for larger studies of the effects of XRT and other sialorrhea interventions on the swallowing and mealtime related quality of life of individuals with ALS.

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P199 A PROSPECTIVE, RANDOMIZED CONTROLLED STUDY COMPARING RADIOTHERAPY VERSUS BOTULINUM TOXIN A AS A TREATMENT FOR DROOLING IN ALS

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Keywords: drooling, treatment, comparison

Background: Drooling is a common and invalidating symptom in patients with Amyotrophic Lateral Sclerosis (ALS). Radiotherapy and botulinum toxin injections are now well accepted as alternative treatment options to the more tradional and often disappointing drug management of this symptom. Still, a well-designed prospective and controlled trial to compare these two methods has never been performed.

Objective: To answer the question which treatment has preference when taking into account effectiveness and side effects.

Methods: In a prospective, randomized, controlled trial we will compare efficacy and tolerability of ultrasound-guided BTX-A injections in parotids and submandibular glands with radiotherapy performed by a single dosage of 7.0Gy bilateral to the parotid glands and the posterior parts of the submandibular glands. To evaluate the effect of treatment measurements will be performed at baseline and after 4, 12 and 36 weeks by the number of used paper handkerchiefs, a drool rating scale, a VAS-scale, ALSAQ-40 and ALS-FRS-revised. Side effects will also be strictly monitored by evaluation of bulbar function.

Since the start of the study eleven patients have been included. Five patients were treated with radiotherapy (RT), five patients with botulinum toxin (BT) injection and one patient with both.

Results: A positive effect to initial treatment was 80% after RT versus 50% after BT. Retreatment of RT raised positive response to 100% and retreatment of BT raised response to 66%. The mean effect size was 48 days after RT versus 45 days after BT (range between one week and 4 months). As a result of both treatments xerostomia and changed viscosity of saliva were mentioned as side effects. No decrease of bulbar functions was observed.

Conclusions: After treatment 10 patients were satisfied for at least a short period of time. No serious side effects were found. The effect of the treatments did not significantly differ in this small group of patients.

P200 CHALLENGE OF HEAD-NECK SUPPORT ORTHOSIS IN AMYOTROPIC LATERAL SCLEROSIS PATIENTS FOR IMPROVING ACTIVITIES OF DAILY LIVING

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Keywords: cervical orthosis, ADL, head-neck support

Background: ALS patients often have problems supporting their own head due to the loss of neck muscle power. With this problem, activities of daily living (ADL) such as transfer, gait, and feeding seem to be prevented. Also, some patients complain about dyspnea and neck pain. From 2002 to 2008, we have tried several types of cervical orthosis (CO). In this study, we would like to report three cases which had a good results in improving ADL after using CO. Also, retrospective research was conducted.

Objectives: Case 1: 64 yrs old, male with bulbar palsy (BP). Though he has a typical orthopedic mold-type CO, he has not used it due to the difficulty in attaching it and neck discomfort.

Case 2: 55 yrs old, female with upper extremity palsy (UEP). Though she was recommended a typical orthopedic soft-type CO, she has not used it because head support is not enough, due to the pressure of her neck and discomfort in the humidity and heat.

Case 3: 72 yrs old, male with bulbar palsy (BP). He is tracheotomy ventilated and bed ridden. He sits in a wheel chair every day for a physical therapy programme.

Methods and Results: Case 1: We measured SpO_2 and heart rate on sitting and after 10 minutes distance. After using CO, better influence was seen for respiration.

Case 2: We compared head position before and after using CO. With CO, she could stand by herself in the short term.

Case 3: We measured SpO_2, heart rate and discomfort before and after using the CO.

Conclusions: Using CO in these above mentioned cases and including the results from our retrospective research, ADL such as sitting, standing and gait of ALS patients are improved. More possibilities of orthoses for motor neuron diseases are should be studied.

P201 TO IMPROVE LIVING WITH ALS/MND: THE PROVISION OF ASSISTIVE DEVICES AND ENVIRONMENTAL ADAPTATIONS

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Keywords: Need, devices, provision

Background: ALS/MND patients need assistive devices and environmental adaptations to remain independent for as long as possible and to limit restrictions in autonomy and participation. In the Netherlands health insurance companies and municipalities provide aids and adaptations needed by ALS/MND patients.

Objectives: The aim of this explorative study was to describe the need for assistive devices and environmental adaptations among ALS/MND patients in The Netherlands and to investigate problems encountered by ALS/MND patients in the process of providing assistive devices and environmental adaptations.

Methods: A questionnaire designed for the purpose of the study was sent to 306 of the estimated 1000–1200 patients with ALS/MND of whom 214 (179 with ALS and 35 with PMA) completed the questionnaire (response rate 68%).

Results: *The need for assistive devices and environmental adaptations:* 82% of the respondents needed one or more devices. 66% ALS/MND patients needed home adaptations in the bathroom, 21% applied for a stairlift and 65% applied for an attendant-propelled wheelchair. 49% ALS/MND patients needed a riser chair, 42% an electric nursing bed and 41% a rollator.

The experienced problems: Major problems identified by ALS/MND patients are: i) The long duration of the process of application and provision (39%). ii) Authorities' lack of knowledge on disease characteristics of ALS/MND and insufficient sympathy for the situation of the individual patient (22%). iii) The bureaucracy and paperwork (15%). iv) Lack of clarity of the legal proceedings (15%).

Five out of 10 ALS/MND patients who needed one or more aids or adaptations experienced problems in the process of providing assistive devices and environmental adaptations.

The two major problems indicated by ALS/MND patients which they considered as being most urgent to be solved are: 1) time delay in the delivery of devices and adaptations and 2) the authorities' lack of knowledge on the disease.

Discussion and Conclusions: The results of this study indicate major problems perceived by ALS/MND patients in the process of providing assistive devices and environmental adaptations.

The bureaucracy, the authorities' lack of knowledge on disease characteristics and the long time delays are the main incumberances to the ALS/MND patient that need to be addressed to reduce patient distress.

The most appropriate measures to be taken are: i) to support ALS/MND patients where needed and to meet their information needs during the process of provision. ii) To increase authorities' knowledge on disease characteristics of ALS/MND.

P202 DISCUSSIONS BETWEEN ALS PATIENTS AND THEIR FAMILIES ABOUT INVASIVE MECHANICAL VENTILATOR USE

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Keywords: decision making, quality of life, qualitative reseach

Background: About 7300 patients were being treated for ALS in Japan in 2007, with a relatively high rate of invasive mechanical ventilator use compared to other countries. The burden of ventilator use on family caregivers is problematic, so making a unilateral decision to use a ventilator is difficult for patients. Understanding what patients and their families think about this decision is thus important.

Objectives: To determine factors related to decision making about ventilator use, ALS patients not using ventilators were interviewed about their thoughts from diagnosis to the present. This study attempted to assess discussions between ALS patients and their families specifically about invasive mechanical ventilator use.

Methods: This qualitative, descriptive study was performed between May and September 2005. Subjects comprised 5 ALS patients satisfying four criteria and 5 family members acting as primary caregivers. Criteria for patients were: 1) knowledge of diagnosis; 2) understanding of the possible need for ventilator use; 3) no current ventilator use; and 4) capacity to tell others about their thoughts.

The 3 main questions examined what patients thought when diagnosed with ALS, changes in daily life as symptoms progressed and thoughts about ventilator use. Data were collated from semi-structured interviews.

Interviews were transcribed, then sentences related to study objectives were extracted to: analyze decision-making processes regarding ventilator use between patients and family caregivers; evaluate family discussions; and ascertain characteristics of each patient/caregiver pair.

Results: Subjects were interviewed once or twice. Mean interview durations for patients and caregivers were 150 and 105 min, respectively. Regarding patient thoughts on ventilator use, one patient was willing, two patients were unwilling and two patients were undecided. In addition, three caregivers wanted to respect patient decisions, one caregiver was undecided and one caregiver did not want a ventilator. Four pairs had not discussed ventilator use, for reasons such as: 1) both parties were undecided; 2) the caregiver was unenthusiastic about ventilator use; 3) the patient had decided against ventilator use; or 4) the caregiver did not want their opinion to affect the decision-making process of the patient.

Discussion and Conclusions: Only one of the 5 pairs had discussed ventilator use. The other 4 pairs had not held such discussions. The pair that had talked about the issue agreed on not using a ventilator. Individual differences were seen in the amount of information and data about ALS, with some subjects worried about making decisions regarding ventilator use, since they could not anticipate disease progression. As decisions regarding ventilator use are life-or-death decisions, some subjects stated how difficult this issue was to talk about and expressed a desire for professional intervention.

In order to make a decision about using invasive ventilation that satisfies both patients and their families, it is important for them, with the support of specialists, to fully discuss the decision on ventilator use.

P203 AT-HOME ARTIFICIAL VENTILATION CARE AND AUTONOMY OF ALS PATIENTS

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Keywords: Home Artificial Ventilation Care, Autonomy, QOL

Objective: To determine what conditions are needed for ALS patients who have undergone medical procedures to continue to live alone, we examined the difference in process of support between a patient who failed to continue at-home artificial ventilation care and a patient who received training at the ALS Care Center of Miyagi National Hospital and has been receiving at-home care.

Patients: Patient 1 is a 53-year-old female who developed ALS in 1995 and was placed under artificial ventilation in 2003, with a total duration of disease of 12 years.

Patient 2 is a 60-year-old female who developed ALS in 2003 and was placed on artificial ventilation in 2006, with a total duration of disease of 5 years.

Methods of support: Patient 1: Based on the patient's wish to receive at-home care, a care manager recruited caretakers, who were trained in aspiration techniques at the hospital, and at-home care was then started. To support the patient's life under at-home care, 8 to 10 caretakers sent from a care agency were always by the patient, working for 24 hours and 365 days per year, on a four-shift per day basis. Shortage of care expenses was covered by the care agency.

Patient 2: Support was provided to encourage the patient to make a care plan for at-home care and make a request to her primary care physician for herself. In addition, she was required to go in and out of the hospital repeatedly so that she was able to gradually develop an environment for at-home care herself while solving problems associated with at-home care.

Results: Patient 2 posted a call for volunteer caretakers in newspapers herself. This activity expanded the network of support and thereby dramatically reduced the burden of care on her family member (daughter), who had been totally responsible for her care. This resulted in consolidation of the basis of the at-home care system, and an environment suitable for at-home artificial ventilation care with 24-hour caretakers has been established.

In contrast, in the case of patient 1, shortage of caretakers occurred because of withdrawal of many of the caretakers due to psychological burden of the difficulty of their work, resulting in shrinkage of the network of support and failure of at-home care.

Discussion: There were marked differences between patients 1 and 2 in methods of support before the start of at-home care. Specifically, patient 1 was not involved in the process of developing an environment for at-home care, and preparation of the care environment was mainly performed by a care manager. In the case of patient 2, support was provided to facilitate the development of autonomy, leading to a willingness on behalf of the patient to improve her own life herself and encouraging her to work positively to develop a care environment herself. This resulted in a difference in quality of the at-home care system between the two patients.

Conclusion: Providing patients with support before the start of at-home care facilitates the development of autonomy, a willingness to improve his/her own life, leading to expansion of the network of support, and consolidation of the basis of the at-home care system.

P204 OUTCOMES OF PATIENTS WITH ALS WHO HAD SEIQOL-DW ASSESSMENTS BEFORE MAKING ADVANCE DIRECTIVES

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Keywords: SEIQoL-DW, ALS, advance directive

Background: We reported previously that some Japanese patients with amyotrophic lateral sclerosis (ALS) wished to control their treatment and management after respiratory or swallowing function deteriorated, especially in regards to the decision to use mechanical ventilation via tracheostomy. Although there is no legislation mandating advance directives in Japan, discussion about patients' involvement in decision making has increased since a presentation of the Guideline for the Process of Decision Making on End-of-Life Care by the Ministry of Health, Labour and Welfare of Japan, in May 2007. The newly presented Guideline is stimulating discussion among doctors who treat patients with serious conditions, but it may also accelerate excessive use of an advance directive documents without assessing patients' thoughts and efforts to improve their own situations. The Schedule for the Evaluation of Individual QOL-Direct Weighting (SEIQoL-DW) is an acceptable tool to evaluate patients' thoughts. While it is still very rare for Japanese patients to have an advance directive, we had some patients who completed a SEIQoL-DW assessment before writing a directive.

Objectives: Our goal was to survey patient outcomes and the index of SEIQoL-DW regarding individuals' needs to improve their care at the time of making advance directives.

Methods: From case records of ALS patients who had follow-ups and were visited more than once at their homes, six domiciliary patients who had advance directives from August 2004 to March 2006 were found. Four of them had a SEIQoL-DW assessment before making an advance directive, and had follow-ups until they died or revoked their directives through March 2008.

Results: There were three male and one female patients, between 38–68 years of age (mean age $=54.3\pm15.0$ years) with a disease duration of 3–6 years (mean duration 4.3 ± 1.3 years). Two patients had died, and two were alive at home. The deceased patients' SEIQoL-DW indices were 60.9 and 68. The two living patients had scores of 11 and 30. One patient was followed up with repeated SEIQoL-DW assessments and showed a remarkable increase of the index from 30 initially to 77.5 on the most recent assessment. This increase was attributed to support provided for improving communication, through which the patient learned computer skills and was able to access the Internet and communicate with friends, family, and healthcare professionals. She revoked her advance directive, and accepted 24 hour mechanical ventilation at home.

Discussion and Conclusions: As the number of subjects was too small to evaluate statistically, the effectiveness of the

assessment requires further investigation; but SEIQoL-DW assessments could reflect the patients' need for their situation to be improved before making advance directives.

P205 STOPPING LIFE PROLONGING RESPIRATORY SUPPORT. REFLECTION ON OUR PRACTICE

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Keywords: withdrawing treatment, respiratory support, reflection

Background: Some patients who use respiratory support decide that they no longer wish for this to continue to prolong there lives. This concerns their perception of their dignity, quality of life and fear of the future. Removing such respiratory support can be physically and emotionally very distressing for the patient as they develop hypoxia, hypercapnia and acute breathlessness. This raises considerable practical, emotional and ethical issues for the health care team. Reflection is an intentional and skilled activity requiring an ability to analyse practice actions and make judgements regarding their effectiveness' (1). At the core of reflective practice is a purposeful contemplation of an experience or event which leads to new ways of thinking or behaving in practice.

Objectives: 1) To develop the safety, efficacy and quality of the care for future patients. 2) To minimise risk for the health care team. 3) To further understanding of this are of clinical practice.

Methods: The specialist palliative care team reflected on two patients who had requested support in stopping their nasal intermittent positive pressure ventilation. The reflection took place both individually and as a group using the Driscoll 'What' model (2). The role of the various health care professionals in working through this decision with the patient, family and carers and the care that was provided as the respiratory support was removed were specifically considered.

Results: We have found that there is no literature to inform our practice. Our reflection identified a breadth of concerns amongst professionals. These include; anxiety about competence in alleviating symptoms of distress within ethical boundaries; a profound challenge to individual practitioners' values which causes stress; a vital need to support each other in the distress related to proximity of administration of sedative medication to relieve distress and the occurrence of death.

Conclusions: The needs of the team who provide the care at the time of withdrawal support must be considered and planned for in advance. Introducing them to the patients care at an earlier stage is of importance.

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P206 ANALYSIS OF THE IMAGE AND CORPOREAL SCHEME AND THE QUALITY OF LIFE OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Key Words: Amyotrophic Lateral Sclerosis, Image and Corporeal Scheme, Quality of Life.

Background: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease creates changes in the self-perception of the patient. Body Image (BI) is a mental representation formed by the body itself, perception, movement and the emotional/affective and libidinal reactions are also part of its development. Analysis of the BI identifies alterations that influence the Quality of Life (QoL), it can be analysed by Drawing the Human Figure (DHF) which reflects the knowledge and the experience of the body itself.

Objectives: 1) To evaluate the image and corporeal scheme of patients diagnosed with ALS to verify possible alterations or distortions, 2) to evaluate the quality of life and the possible correlations between the many dimensions of the 40 item Amyotrophic Lateral Sclerosis Assessment Questionnaire (ALSAQ-40) and the DHF.

Methods: Seventeen patients with ALS participated in this research answering the questionnaire ALSAQ-40 and completing the DHF, which was evaluated quantitatively and qualitatively. The Spearman correlation coefficient was used to verify the relationship between the alterations in self-perception and the dimensions of ALSAQ-40.

Results: The corporeal image and all the dimensions of the ALSAQ-40 was altered in all seventeen patients with ALS, that being the case, many Mobility problems became evident in relation to Emotional function. There is a correlation between the DHF and the Eating and Drinking dimensions (ALSAQ-40) besides the many direct correlations between the dimensions themselves.

Discussion: The results correlated with the literature, showing that the Emotional functioning dimension (ALSAQ-40) did not present itself as the most altered. Although, during the evaluation of the DHF, it was possible to observe projections of feelings like preoccupation, fear, anxiety, sadness, escaping from reality and restlessness in the spheres of sexuality and family contradicting what was presented in the questionnaire. While the ALSAQ-40 is a specific and self-applicable instrument to evaluate the QOL of the patients with ALS, the execution of the DHF allows the patients to project themselves subconsciously.

Conclusion: The individual results obtained through the use of both instruments show the need for more attention to be given to the self-perception of patients, as QoL is directly related to self-image and self-image has a strong emotional component. The use of DHF presents the most vulnerable points of the patient, giving the therapist conditions to assist the patient with ALS in a more systematic and humanized way.

P207 HEALTH STATE AND EMOTIONAL IMPACT OF THE DISEASE IN SPANISH PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Keywords: ALSAQ-40, Quality of Life, Emotional Impact

Background: ALS has a high emotional impact on the patients, an impact that probably affects their quality of life, in different ways during the disease. The ALS Assessment Questionnaire 40 (ALSAQ-40) is used to evaluate the patient's health state in five scales: Physical Mobility, Activities of Daily Life (ADL)/Independence, Eating and Drinking, Communication, and Emotional Functioning. The Spanish version has been validated by us previously.

Objectives: To analyze the emotional impact of the disease in the established different areas of the ALSA-40 along the course of the disease. To determine which disabilities and when produce higher emotional distress.

Methods: A descriptive and longitudinal study was conducted over 4 years on perceived health status in 231 ALS patients. The ALSAQ-40 was used at three different stages: after diagnosis, at a time when their disability had progressed, and at the terminal stage. All patients were diagnosed with definite or probable ALS by the El Escorial Diagnostic Criteria.

Results: In the Spanish patients there was not a direct correlation between grade of severity of the disease and emotional impact. The deepest feeling of depression occurred more frequently around the time of diagnosis. At the first evaluation, 20% of patients felt "frequently" depressed and 17% were "never" depressed versus 12% and 30% respectively at the third evaluation. Only 31% of the variations were in the Emotional Functioning scale are correlated significantly with the variable physical state. The progressive reduction in ADL/Independence is the single factor most related to depression. There were not significant feelings of loneliness, boredom, shame or hopelessness. Hopelessness about the future decreased with the course of the disease while increases concerned the future consequences of the disease.

Conclusions: Psychological support around the time of diagnosis is advisable to overcome depression. Psychotherapy along the course of the disease must take into consideration the emotional impact of the progressive loss of autonomy and the fear of future consequences of the disease.

P208 END OF LIFE CARE OF PEOPLE WITH MND/ALS – A SURVEY OF THE MEDICATION USED IN SPECIALIST PALLIATIVE CARE

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Keywords: palliative care, opioids, end of life

Background: Palliative care is increasingly provided for people with MND/ALS. This often starts soon after diagnosis as there is no curative treatment for the disease. The final stages of the progression of the disease are often difficult with increasing symptoms and issues for patient, family and professional carers (1). Professional carers often fear the use of medication, in particular opioids (2).

Objectives: This study aimed to show the medication used within specialist palliative care in the end of life care for people with MND/ALS.

Methods: A questionnaire was developed to record medication given to patients with MND/ALS in the last 72 hours of life, together with basic information about the patient and disease progression. Six hospices in the UK and Ireland agreed to complete the details of the last 10 patients who had died under their care in the hospice.

Results: 60 patients were analysed and the results show that medication is usually necessary in the control of symptoms at the end of life. These are commonly morphine as analgesic, midazolam as sedative and muscle relaxant and glycopyrronium bromide to reduce lung secretions. The doses of the medication are not greater than the doses used in the palliative care of other patient groups, such as those with cancer, or advancing respiratory or cardiac disease.

Discussion: Many patients with MND/ALS, their families and carers and often the professional carers fear the final stages of the progression of MND/ALS – fearing in particular pain, dyspnoea and choking (1,3). These results show that medication can be used effectively in the management of the symptoms experienced at the end of life and the doses are not different to those used in the care of other patient groups. Health professionals can be reassured that after appropriate symptom assessment the careful use of opioids, and other medication, is helpful and appropriate at the end of life.

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P209 SYMPTOMS AND UNBEARABLE SUFFERING IN PATIENTS REQUESTING EUTHANASIA OR PHYSICIAN-ASSISTED SUICIDE: ARE THERE DIFFERENCES BETWEEN ALS, HEART FAILURE AND CANCER?

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Keywords: euthanasia, cancer, heart failure

Background The percentage (20%) of patients with amyotrophic lateral sclerose (ALS) that die after euthanasia or physician-assisted suicide (EAS) is higher compared to most other terminal diseases in the Netherlands including cancer (5%) and heart failure patients (0.5%). One of the mandatory conditions in the Dutch law for performing EAS is that the patient requesting EAS suffers unbearably. Palliative care should prevent this unbearable suffering if possible. It is unclear why the rate of euthanasia in ALS is higher compared to other terminal diseases, therefore more insight in the disease specific unbearable suffering, which causes patients to prefer death over life is desirable.

Objectives: In this research we studied whether ALS patients have different reasons for requesting EAS compared to cancer and heart failure patients.

Methods: Dutch physicians of patients with a request for EAS can consult an independent EAS specialized physician (SCEN physician). This SCEN physician visits the patient and reports the patients' reasons for unbearable suffering that are the motives for requesting EAS. For this study, the treating physicians who consulted the SCEN physicians, mostly general practitioners, were asked to fill out a questionnaire on sex, age, diagnosis and physical and depressive symptoms of the patients requesting EAS.

We collected all (n = 3891) SCEN reports in the Netherlands between April 2000 and the end of 2002. Next, we selected all (n = 51) SCEN reports of ALS patients. To compare their reasons of unbearable suffering we also selected all (n = 61) heart failure patients and made a random selection of the cancer patients (n = 73). We collected and compared questionnaires from the treating physician on the patients' physical and mental symptoms during their request for EAS.

Results: The top three most reported reasons for unbearable suffering in ALS patients were fear of suffocation (45%), feeling dependent (29%) and loss of dignity (20%). The top three of cancer patients was pain (46%), fatigue (28%) and loss of dignity (24%). The top three of heart failure patients was dyspnoea (52%), dependency on others (37%) and knowing that the suffering will only get worse (30%). According to the treating physician ALS patients had less frequent fatigue (OR 0.169, 95% CI 0.043–0.658), pain (OR 0.265, 95% CI 0.119–0.591), nausea (OR 0.106, 95% CI 0.042–0.270) and vomiting (OR 0.093, 95% CI 0.028–0.307) than cancer patients at the time of the EAS request and poor appetite less frequently (OR 0.183, 95% CI 0.051–0.653), dyspnoea (OR 0.242, 95% CI 0.081–0.723) or nausea (OR 0.338, 95% CI 0.115–0.994) compared to heart failure patients.

Cancer and heart failure patients reported significantly more frequent somatic complaints as a reason for EAS than ALS patients ($P < 0.001$).

Conclusions: ALS patients most often mention psychosocial reasons for requesting EAS. Compared to cancer and heart failure patients, somatic complaints are significantly less mentioned.

P210 INTERACTING WITH OTHER PEOPLE LIVING WITH ALS/MND: PATIENT AND CARER ATTITUDES TO SUPPORT GROUPS

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Keywords: Support groups, personal experiences, qualitative research

Background: As ALS/MND is rare, general understanding of its impact on people's lives remains limited. Support groups, both face-to-face and online, offer contact with others who understand at first-hand what living with MND is like. These interactions can provide practical advice and emotional support. However, it is unknown why some people choose not to get involved.

Objectives: To explore the range of individual needs and attitudes to support groups amongst people living with ALS/MND and carers; to understand what motivates people to get involved or not; to develop recommendations to help professionals and voluntary groups in organising support groups.

Methods: This paper draws on narrative interview data collected for two research projects in the UK. The collective sample includes 48 patients living with ALS/MND and 22 carers, interviewed between 2005 and 2007. Data about attitudes to and needs for support groups will be subjected to a secondary analysis, using a coding framework based on both anticipated and emergent themes, and refined using constant comparison and deviant case analysis. This will be conducted by the two researchers responsible for the original research, making this an innovative approach to data sharing and re-analysis. Ethical approval is already in place.

Results: Preliminary observation of the data suggests several issues to be explored. Positive advantages of face-to-face and online support groups include practical advice (e.g. on welfare benefits and equipment). Many people draw comfort from talking to others who understand the range of emotions they experience.

Seeing others managing with more severe disability can provide an encouraging example, but can also create anxiety. Equally, some people with slow progression worry that their presence at support groups could upset others with more rapid progression. Deaths of fellow group members are saddening and depressing. Some people avoid support groups because they do not want their identity and social life to be defined by the condition.

Practical difficulties (e.g. timing and transport) affect some people's attendance of face-to-face groups, whilst lack of IT skills or computer adaptations may affect involvement in online groups.

Some people living with ALS/MND feel face-to-face groups are inappropriately dominated by carers and volunteers, whereas some carers feel unable to talk about issues that concern them when people living with the condition are present. Online support groups can offer a more indirect way of interacting with others, but some users are still upset by photographic images posted with messages.

Discussion: There has been little research into attitudes to support groups for ALS/MND. Greater understanding of the range of reactions that people have to support groups, their hopes for what they may gain, and their anxieties about what

they may encounter can help those involved in organising groups to improve their appropriateness and accessibility.

P211 TRANSFORMATION OF RELATIONSHIPS FOLLOWING A DIAGNOSIS WITH ALS/MND

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Keywords: relationships, narratives, changes

Background: Health professionals are increasingly attempting to understand illness from the patient's perspective. Making use of illness narratives is one way of becoming more informed about the personal experience of living with illness.

Objectives: To explore the personal experience of living with ALS/MND as documented in personal illness narratives, written by people diagnosed with the illness.

Methods: A detailed search strategy loosely based on systematic review methodology was adopted to identify illness narratives in print and internet form written by people diagnosed with ALS/MND. The 161 narratives identified were subject to content and thematic analysis. Data management was aided by Nvivo 7 software.

Results: A number of key themes emerged. This paper focuses on the transformation of relationships at a number of levels following a diagnosis of ALS/MND. Authors comment that they endure changes in their relationships within the family and an altered dynamic within the domestic setting. This is particularly apparent with considerations of gender, where individuals are no longer able to perform 'usual' gender specific activities. There is evidence of inversion of relationships as changes occur within generational roles as individuals develop dependent relationships on others. Numerous narratives, regardless of genre, document authors' changing relationships with their own bodies. There is a strong impression of accelerated aging and the use of powerful imagery in an attempt to capture the impact of the disease at a personal level. For some, defined by their occupation, there is loss of identity when employment ends. Changes within the immediate environment result in the loss of individuality and private space.

Many authors convey their sorrow at the loss of their ability to initiate physical contact, particularly with their spouse/partner. Relationships are also affected by a loss of intimacy between husband and wife. The disabilities caused by ALS/MND engender a sense of vulnerability in some people at having to rely on others for their needs.

Discussion and Conclusion: What we have here is authors writing about the various changes forced on them as a result of their illness. What emerges is a sense of attempts to overcome adversity and re-evaluate life in light of current circumstances. These illness narratives provide an insight into the effects of the disease on the individual diagnosed with ALS/MND. They also provide a glimpse into the world of other people from the perspective of the ill person. As such, the findings have implications for the training of health care professionals involved in the care of people with ALS/MND.

P212 'ON THE INTERNET YOU DON'T NEED FEET': USING TECHNOLOGY TO OVERCOME CHANGES IN RELATIONSHIPS WITH SOCIETY FOLLOWING A DIAGNOSIS OF ALS/MND

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Keywords: narratives, internet, society

Background: Recording personal experiences of ill health within an illness narrative enables individuals to document how their condition has impacted on their lives and helps to illuminate the steps they have taken to overcome adversity.

Objectives: To gain an understanding of the role of the internet in the personal experience of living with ALS/MND.

Methods: 161 print and internet narratives identified as a result of a detailed systematic search strategy were subject to content and thematic analysis. Data management was aided by Nvivo 7 software.

Results: A number of key themes emerged. This paper focuses on the use of modern technology by people diagnosed with ALS/MND in their attempts to cope with the disease. Being isolated pervades many narratives, as authors retreat from their everyday activities. The emergence of the internet and the availability of chat rooms and discussion groups specifically for people with ALS/MND have opened up numerous opportunities for interaction with the outside world. This has helped to ease the sense of isolation experienced by many authors as their condition deteriorates. New virtual friendships develop as traditional friendships fall away. Those authors experiencing dysarthria comment on how it removes them from spontaneous interaction with others. Communication becomes a matter of need rather than a social exchange. An inability to convert one's thoughts into the spoken word results in a sense of increasing isolation from the world. Accessing the internet allows authors to overcome their disabilities, even if only momentarily.

Discussion and Conclusion: Illness narratives about life with ALS/MND allow individuals to document how they have adjusted to the effects of the disease. These findings indicate that, despite their disabilities, those diagnosed with the illness can continue to involve themselves within society on a number of levels through accessing the internet.

P213 COMPARING OUTCOMES OF PATIENTS WITH ALS IN THE COMMUNITY VS LARGE ALS CENTERS, INSIGHTS FROM THE WEB-BASED ALS PATIENT C.A.R.E DATABASE

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Keywords: Outcomes, registry, diagnosis

Background: Data on outcomes for patients cared for in large ALS centers comes from the ALS C.A.R.E database.

Objective: To compare outcomes of patients treated in the community compared to large specialized ALS clinics.

Method: Treatment and outcomes were compared from the ALS C.A.R.E database with data entered in the web-based,

patient-driven database, ALS Connection, launched in January 2006. This analysis compares 82 patients enrolled in ALS Connection cared for in community practices (SOLO) with 4971 patients enrolled in the ALS C.A.R.E database (C.A.R.E), cared for in large multidisciplinary ALS clinics.

Results: SOLO patients were generally more advanced than C.A.R.E patients (limited in most activities 77% SOLO, vs 20% C.A.R.E).

More SOLO patients (56%) than C.A.R.E patients (21%) were unsatisfied with the way they were told about their diagnosis. More SOLO patients felt that their diagnosis was told to them in an insensitive manner (16% SOLO vs 3% C.A.R.E), that they were given too little information about the disease (20% vs 5%), and that they were given no sense of hope (22% vs 5%). 69% of C.A.R.E patients were given information about ALS agencies such as MDA and ALSA, compared to only 37% of SOLO patients.

Regarding therapeutics, fewer SOLO patients utilized therapists: speech therapist (29% SOLO vs 63% C.A.R.E); nutritionist (29% SOLO vs 66% C.A.R.E), social worker (22% vs 65%), home nursing (22% vs 65%)or psychologist (16% vs 66%).

Although a similar percentage of SOLO & C.A.R.E (60% vs 54%) patients have seen a physical therapist, 22% of SOLO patients who have tried physical therapy found it unhelpful, whereas only 2% of C.A.R.E patients felt the same way. The same is true for occupational therapy. 60% of C.A.R.E patients have seen an occupational therapist, and only 2% of those found it unhelpful. 45% of SOLO patients have tried OT, and 19% of those who tried thought it was unhelpful.

More Web patients got most information regarding ALS from the internet (53% SOLO vs 9% C.A.R.E). More patients were satisfied with their care in the ALS C.A.R.E database (70% SOLO vs 91%C.A.R.E).

Conclusion: Patients with ALS who enrolled in a web-based registry were less satisfied with care, had a less satisfactory experience in learning about the diagnosis, less access to symptomatic therapy, and less satisfactory experiences with therapy, than those in ALS C.A.R.E. Further research is needed to identify the reasons for these differences and how stable these differences are.

P214 STIMULATING INNOVATION TO IMPROVE THE QUALITY OF LIFE IN ALS VIA A DESIGN CHALLENGE: EXPERIENCES AND DIRECTIONS

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Keywords: assistive technology, innovation, students

Background: Technical advances to assist patients with ALS have great potential for enhancing their quality of life. Unfortunately, technological innovations have been slow in development and have not kept pace with other advances in the care and understanding of ALS. We believe that innovation can be accelerated significantly by increasing the familiarity of biomedical engineers, human factors experts and computer scientists with the challenges faced by people with ALS and their care team.

Objectives: To encourage the development of innovative technologies to improve quality of life for patients with ALS through collaboration with young engineers, computer scientists and technologists in academic settings and highlight the compelling opportunities for creative exploration and prototyping

Clinical Description: Through the ALS Society of British Columbia, Canada, a prize was offered to students at undergraduate and postgraduate levels of engineering, including the fields of biomedical engineering, electrical and computer engineering, mechanical engineering and engineering science, to design innovative technologies that would assist and improve the quality of life of ALS patients. Prioritized areas of need were first identified by the ALS Society through surveys and interviews involving ALS patients and caregivers, as well as members of professional ALS care teams. These were grouped into four general areas: mobility, communications, breathing, and feeding. Students were encouraged to initially meet directly with patients, with appropriate mentoring and supervision, to learn more about the identified needs and to develop potential design solutions through adaptation of existing devices or development of new technologies. The resulting design projects were completed by six teams at three universities and a college over the course of an academic year. The results of four projects were suitable for live demonstration involving ALS patients, caregivers and a multidisciplinary evaluation panel: 1) A dynamic, versatile neck brace that whilst supporting the head and neck allows for ready movement of the neck in all directions, and stabilizes the head and neck for vehicular travel; 2) An eye gaze tracking system to enhance communication in patients with severe dysarthria or unintelligible speech; and 3) Integration of breathing and voice technology for improved communications in BiPAP dependent patients during the later stages of ALS.

Recommendations: A modest prize for innovative technology has stimulated potentially novel methods to improve quality of life for ALS patients, develop collaborations between young engineers and technology developers, physicians and the ALS Society of BC. It has also introduced ALS to those seeking rewarding career opportunities that involve the development and adaptation of innovative technologies. The initially developed technologies themselves have raised important questions in and of themselves which will result in further improvements.

P215 AN EEG IMPEDANCE CORRELATION STUDY FOR BRAIN COMPUTER INTERFACE

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Keywords: brain-computer interface (BCI), electroencephalography (EEG), communication

Background: Amyotrophic lateral sclerosis (ALS) is characterized by progressive weakness resulting in a state of profound disability including the loss of functional speech. The rise of new technologies allows people living with ALS and other individuals with severe motor disabilities to communicate as well as access the computer using alternate methods. One alternative access and communication method is an Electroencephalographic (EEG)-based brain-computer interface (BCI). The EEG-based BCI allows individuals with severe disabilities to communicate using a computer even when other conventional devices have failed, as the system is independent of neuromuscular activity. Impedance is a critical factor in the optimum performance of electrical systems and current literature correlates lower impedance with superior EEG recordings. However, no systematic study examines the relationship between BCI performance and impedance. The

need to obtain low impedance may require significant additional time and abrading the skin may result in patient discomfort. Therefore, striking a reasonable balance between impedance and BCI performance is critical to the successful implementation of the BCI.

Objectives: The objective of this study was to determine the correlation between BCI performance and EEG impedance to determine the upper limit of impedance which allows optimal performance with BCI in a practical and clinically relevant paradigm.

Methods: The subjects have an electrode cap placed on their head to record their EEG. A small amount of gel is applied to each electrode. Impedance measurements are taken. Each subject is requested to attempt to spell a sequence of words using the P300-based BCI speller while the EEG is being recorded. This spelling sequence is then repeated 5–10 times after reducing the impedance. The impedance values, number of sequences, time, and performance percentages are recorded. Overall, the subjects attend 2 sessions during which the EEG and impedance measurements are recorded.

Results: In the three subjects (two ALS and one control) studied to date, data indicates a correlation between impedance and BCI performance. High impedance values result in poor spelling performance. In our subjects, adequate performance was obtained with up to an average value of 20 kohm impedance. Further testing will expand these results and fully determine the upper limit of acceptable impedance that still allows functional use of BCI technology in order to make the technology acceptable and practical for patients and caregivers.

Discussion and Conclusions: Current P300-based BCI protocols suggest that each electrode must have impedance values below 10 kohm for optimum performance. However, in subjects studied to date, we have found that BCI protocols may be amended to use impedance values as high as 20 kohm, resulting in a time savings and easier application for the caregiver. This study will help to translate BCI to practical use for ALS patients with severe motor and speech limit.